

THE
BOTANICAL MAGAZINE

PUBLISHED
BY
THE BOTANICAL SOCIETY OF JAPAN

Volume 74

Nos. 871-882

TOKYO

1961

植 物 学 雜 誌

第 74 卷 871—882 号

日 本 植 物 学 会 発 行

東 京

1 9 6 1

AUTHOR INDEX

Articles

- FUJII, T., and ISHIKAWA, S.: The Effects of Temperature after the Light-exposure on the Germination of *Oenothera* Seeds 414
- FUJII, M. (→HORI, T.)
- FUJII, T. (→ISHIKAWA, S.)
- FUJIO, M. (→KIMURA, K.)
- HANAWA, J.: Experimental Studies on Leaf Dorsiventrality in *Sesamum indicum* L. (Jap. with Eng. Summary) 303
- HANAWA, J.: Further Studies on the Double-leaf Formation in *Sesamum indicum* L. 402
- HASHIMOTO, T.: Synergistic Effect of Indoleacetic Acid and Kinetin on the Primary Thickening of Pea Stem Segments 110
- HATAKEYAMA, I. (→KAWANO, K.)
- HIDA, M., and IDA, K.: Studies on Carotenoids of Green and Autumnal Red Leaves of *Metasequoia glyptostroboides* (Jap. with Eng. Summary) 369
- HIDA, M.: The Comparative Study of Taxodiaceae from the Standpoint of the Development of the Cone Scale (Supplement). The Development of *Metasequoia's* Cone Scale (Jap. with Eng. Summary) 449
- HIDA, M.: Studies on the Bark of *Metasequoia glyptostroboides*, with Special Reference to Giant Cork Cells (Jap. with Eng. Summary) 514
- HIROMOTO, K.: Culture of Mycelia from the Fruit Body of Hymenomycetes by "The Hymenium-Isolation Method" (Jap. with Eng. Summary) 154
- HIROMOTO, K.: A discussion on the Taxonomy of *Tricholoma aggregatum* (Schaeffer ex Secretan) (Constantin et Dufour and *Tricholoma conglobatum* (Vittadini) Saccardo (Jap. with Eng. Summary) 419
- HORI, T., and FUJII, M.: On the Growth Substances Economy before and after Flowering in Each Organ of *Portulaca grandiflora* Hook. 357
- HUZISIGE, H., and SATOH, K.: Photosynthetic Nitrite Reductase I. Partial Purification and Properties of the Enzyme from Spinach Leaves 178
- ICHIMURA, S.: On the Spatial Difference of the Primary Production in the Lake and its Relation to Environmental Factors 6
- IDA, K. (→HIDA, M.)
- INO, S. (→NISHIBAYASHI, T.)
- INOUE, H.: Supplements to the Knowledges on *Takakia lepidozoides* Hatt. et Inoue (Jap. with Eng. Summary) 509
- ISIKAWA, S., FUJII, T., and YOKOHAMA, Y.: Photoperiodic Control of the Germination of *Eragrostis* Seeds 14
- ISIKAWA, S. (→FUJII, T.)
- ITAGAKI, S., KOBATA, M., and KINOSHITA, S.: Cytological Studies on *Micrococcus glutamicus*. Part V. The Effects of Na-citrate and Na-malate on Cell Elongation and Branching (Jap. with Eng. Summary) 452
- ITAGAKI, S., KOBATA, M., and KINOSHITA, S.: Cytological Studies on *Micrococcus glutamicus*. Part VI. Investigations of Substances which have the Effects of Cell Elongation and Enlargement (Jap. with Eng. Summary) 498
- ITO, T.: Fruit Body Formation of Red Bread Mold *Neurospora crassa* IV. Effects of Ammonium and Nitrate Ion in the Medium on Size of the Perithecium 379
- IWAKI, H. (→NOMOTO, N.)
- IWASAKI, N.: Entwicklungsgeschichtliche Untersuchungen an den Characeen I. *Nitella inokasiraensis* 211
- IZAWA, M.: Effect of Indoleacetic Acid on Protein and Ribonucleic Acid Syntheses in Cultured Bean Germ-axes 98

IZAWA, M.: Water Uptake and Indoleacetic Acid Destruction of Cultured Bean Germ-Axes	169
KATO, I.: Studies on the Phosphorylase Action in Plants I. Histochemical Investigation of Phosphorylase in Growing Leaf Epidermis	297
KATSUMI, M.: Studies on the Mode of Action of 1:4-dihydronaphthoic acid-1 I.	436
KAWANO, K., TUZII, R., and HATAKEYAMA, I.: Seasonal Changes in the Osmotic Value, Water Content and Solute Ratio of the Bud of Mulberry Tree	118
KIKUCHI, M.: Studies on Pigment Formation in <i>Penicillium islandicum</i> Sopp., NRRL 1036 during Cultivation (Jap. with Eng. Summary).....	42
KIKUCHI, M., and NAKAHARA, M.: Sequence of Pigment Formation in the Mycelia of Defective Strains Obtained from <i>Penicillium islandicum</i> Sopp. by UV-irradiation (Jap. with Eng. Summary).....	463
KIMURA, K., and FUJIO, M.: Variation in the Expansibility of Pilei in <i>Coprinus macrorrhizus</i> form. <i>microsporus</i> (Jap. with Eng. Summary).....	259
KIMURA, K.: Effect of Temperature and Nutrients of Flower Initiation of <i>Raphanus sativus</i> L. in Total Darkness	361
KINOSHITA, S. (→ITAGAKI, S.)	
KOBATA, M. (→ITAGAKI, S.)	
KOBAYASHI H.: Chlorophyll Content in Sessile Algal Community of Japanese Mountain River	228
KOBAYASHI, H.: Productivity in Sessile Algal Community of Japanese Mountain River	331
KOYAMA, T.: Concerning the Variants of the <i>Carex sachalinensis</i> Group. Taxonomic Studies of Cyperaceae 13.....	321
KURITA, S.: Chromosome Numbers of Some Japanese Ferns (II)	395
KUWAYAMA, Y., SHO, S., and USAMI, S.: Cyanide-Stimulation of <i>Lysichiton</i> Spadix Respiration (Jap. with Eng. Summary).....	375
MASUBUCHI, N.: Studies on Graft Hybrids in Solanaceae I. Chimeras from the Grafting between <i>Salanum nigrum</i> and <i>Lycopersicum esculentum</i> (Jap. with Eng. Summary)....	34
MEHTA, S. C., and VENKATARAMAN, G. S.: Fine Structure of Diatom Valves III.....	248
MONSI, M. (→NOMOTO, N.)	
MURAKAMI, Y.: Paper-chromatographic Studies on Change in Gibberellins during Seed Development and Germination in <i>Pharbitis Nil</i>	241
NAKAHARA, M. (→KIKUCHI, M.)	
NAKAMURA, K.: An Ascospore Color Mutant of <i>Neurospora crassa</i>	104
NAKAO, S.: Effect of Low Temperature on Bacterial Oxidations	60
NAKAZAWA, S.: Developmental Mechanics of Fucaceous Algae XVII. Differential Destruction of the Cortical Layer of Cytoplasm in Ultracentrifuged <i>Coccophora</i> Eggs	1
NAKAZAWA, S.: Developmental Mechanics of Fucaceous Algae XVIII. Localization of Protoplasmic Elements in the Developing Rhizoid.....	161
NAKAZAWA, S.: Developmental Mechanics of Fucaceous Algae XIX. Negation to Presence of Polar Cyto-skeletal Systems in the Endoplasm of <i>Coccophora</i> Eggs	431
NIMURA, H. (→SUZUKI, S.)	
NISHIBAYASHI, T., and INOH, S.: The Formation of Zoospores in <i>Chorda filum</i> (L.) Lamour. (Jap. with Eng. Summary)	195
NISHIDA, Y., and SAITO, S.: Studies of the Germination of the Spore in Some Mosses II. <i>Diphyseium fulvifolium</i> Mitt. and <i>Sphagnum cuspidatum</i> Ehrh.	91
NISHIGAMI, K.: The Effects of 2,4-Dichlorophenoxyacetic Acid on Growth and Respiration in Yeasts	190
NOMOTO, N., IWAKI, H., and MONSI, M.: Physiological and Ecological Analyses of Shade Tolerance of Plants I. Growth of Green-grams under Varying Light Intensities.....	386
OSHIMA, Y.: Ecological Studies of Sasa Communities I. Productive Structure of Some of the Sasa Communities in Japan	199
OSHIMA, Y.: Ecological Studies of Sasa Communities II. Seasonal Variations of Productive Structure and Annual Net Production in Sasa Communities.....	280

OSHIMA, Y.: Ecological Studies of Sasa Communities III. Photosynthesis and Respiration of <i>Sasa kurilensis</i>	349
OSHIMA, Y.: Ecological Studies in Sasa Communities, IV. Dry Matter Production and Distribution of Products among Various Organs in <i>Sasa kurilensis</i> Community	473
OSUMI, M.: Electron-microscopical Studies on Chromoplast I. The Ultra-structure of Chromoplast in Orange.	165
SAEKI, T.: Leaf Growth as Influenced by Dry Matter Production.....	70
SAEKI, T.: Analytical Studies on the Development of Foliage of a Plant Community	342
SAITO, S.: (→NISHIDA, Y.)	
SAKAI, E. (→SHIBATA, M.)	
SATOH, K. (→HUZISIGE, H.)	
SHIBATA, M., and SAKAI, E.: Concerning the Anthocyanins of Two Garden Varieties of <i>Tulipa Gesneriana</i>	186
SHO, S. (→KUWAYAMA, Y.)	
SUGIURA, M.: A Study on Respiration of Bean Seed Embryo.....	19
SUZUKI, S.: The Seasonal Changes of Aquatic Fungi in Lake Bottom of Lake Nakamura (Jap. with Eng. Summary)	30
SUZUKI, S., and NIMURA, H.: Relation between the Distribution of Aquatic Hyphomycetes in Japanese Lakes and Lake Types.....	51
SUZUKI, S.: The Diurnal Migration of Zoospores of Aquatic Fungi in a Shallow Lake (Jap. with Eng. Summary)	138
SUZUKI, S.: The Vertical Distributions of the Zoospores of Aquatic Fungi during the Circulation and the Stagnation Periods (Jap. with Eng. Summary).....	254
SUZUKI, S.: Distribution of Aquatic Phycomycetes in Some Inorganic Acidotrophic Lakes in Japan	317
SUZUKI, S.: Culture Experiments on the Relation of Lake Bacteria to Lake Type.....	494
TAKAMI, W.: Physiological Studies of Pollen, Chiefly, the Effect of Trace Elements on the Germination of Pollen Grains (Jap. with Eng. Summary)	142
TAKEHISA, S.: Anisomaty in the Leaves of Diploid <i>Petunia</i>	494
TAKEMURA, E.: Morphological and Cytological Studies on Artificial Hybrids in the Genus <i>Lycoris</i> . I. On the F ₁ Hybrid between <i>L. sprengeri</i> Comes. and <i>L. straminea</i> Lindl. (Jap. with Eng. Summary)	524
TATEBE, T.: Physiological Studies on the Fertilization in <i>Lythrum salicaria</i> Linn. I. Presence of Pollen-germination Inhibitors in the Ovary	291
TERASAKI, Y.: On <i>Spirillum putridiconchylum</i> nov. sp.	79
TERASAKI, Y.: On Two New Species of <i>Spirillum</i>	220
TOYODA, K.: Changes in Ascorbic Acid Content in Plumules of <i>Nelumbo nucifera</i> During Maturation of the Fruit	28
TSUBO, Y.: Sexual Reproduction of <i>Chlamydomonas</i> as Affected by Ionic Balance in the Medium	442
TSUBO, Y.; Effect of the Supernatant of Illuminated Culture on Dark Mating in <i>Chlamydomonas moewusii</i> var. <i>rotunda</i> (Jap. with Eng. Summary)	519
TUZIL, R. (→KAWANO, K.)	
URAYAMA, T.: Stimulative Effect of Certain Specific Bacteria upon Mycelial Growth and Fruit Body Formation of <i>Agaricus bisporus</i> (Lange) Sing.	56
USAMI, S. (→KUWAYAMA, Y.)	
VENKATARAMAN, G. S. (→MEHTA, S. C.)	
WADA, K.: Studies on the Flower Initiation of Spring Wheat in Sterile Culture I. Effects of Photoperiods and Photo-sensitive Growth Stage.....	236
WATANABE, K.: Studies on the Germination of Grass Pollen II. Germination Capacity of Pollen in Relation to the Maturity of Pollen and Stigma	131
YAGISHITA, N.: Studies on Graft Hybrids of <i>Capsicum annuum</i> L. I. Variation in Fruit Shape Caused by Grafting and the Effects in the First and Second Seed Generations ..	122
YAGISHITA, N.: Studies on Graft Hybrids of <i>Capsicum annuum</i> L. II. Variation in Fruit	

Shape Caused by Grafting for Three Successive Generations	480
YAMAMOTO, M.: Cracking of Bean Seeds Soaked in Water.....	273
YOKOHAMA, Y. (→ISHIKAWA, S.)	

Short Communications

HIRATA, T., and FUJITA, K.: On Amino Acid Constitution of <i>Poncirus</i> Seed Protein	313
MURAKAMI, Y.: Formation of Gibberellin A ₃ Glucoside in Plant Tissues	424
NAKAMURA, T., ISHII, H., and YAMAKI, T.: The Intracellular Localization of Native Auxin in <i>Avena</i> Coleoptile.....	532
TOYOKUNI, H.: Separation de <i>Comastoma</i> , genre nouveau, d'avec <i>Gentianella</i>	198

Miscellaneous Notes

HARA, N.: Apical Views of Vegetative Shoot Apex	267
INOUE, H., and KITAGAWA, N.: <i>Eremonotus</i> (Hepaticae), a New Addition to the Hepatic Flora of Japan.....	266
SAMPATH, S.: Notes on the Taxonomy of the Genus <i>Oryza</i>	269
TOYODA, K.: A Complementary Note on the Chlorophylls in Some Spermatophytic Seeds..	310
URAYAMA, T.: On the Triangular Wedge-Shaped Bamboo Piece Spawns of <i>Lentinus edodes</i> (Berk.) Sing.....	48

Current Literatures	87, 315
---------------------------	---------

Proceedings of the Society	50, 88, 160, 271, 316
----------------------------------	-----------------------

Book Review	86
-------------------	----

索引

(アイウエオ順)

論 説

- 井沢三生: ミトリササゲ培養胚におけるたん白質およびリボ核酸合成に及ぼすインドール酢酸の影響 (英文) 98
- 井沢三生: ミトリササゲの培養胚における吸水とインドール酢酸分解 (英文) 169
- 石川茂雄・藤伊 正・横浜康継: カゼクサ種子の発芽における光週期的処理 (英文) 14
- 石川茂雄 (→藤伊 正)
- 井田和子 (→肥田美知子)
- 板垣史郎・木幡 守・木下祝郎: *Micrococcus glutamicus* の細胞学的研究
第5報 Na-citrate および Na-malate の伸長肥大分岐効果について 6
- 板垣史郎・木幡 守・木下祝郎: *Micrococcus glutamicus* の細胞学的研究
第6報 細胞伸長肥大効果物質について 6
- 市村俊英: 同一湖沼の異なった場所における基礎生産のちがい (英文) 6
- 伊藤太郎: アカパンカビの子実体形成 IV. 培地中におけるアンモニウムと硝酸イオンの被子器の直径に対する影響 (英文) 379
- 井上 浩: ナンジャモンジャゴケに関する知見補遺 379
- 猪野俊平 (→西林長朗)
- 岩城英夫 (→野本宣夫)
- 岩崎尚彦: シャジクモ科植物の生長点の分化と器官形成 I. *Nitella inokasiraensis* (独文) 211
- 宇佐美正一郎 (→桑山弥寿男)
- 浦山隆司: ハラタケ (マッシュルーム) の菌糸生長と子実体形成におよぼす細菌の刺激効果 (英文) 56
- 大島康行: ササ群落の生態学的研究 I. 数種のササ群落の生産構造 (英文) 199
- 大島康行: ササ群落の生態学的研究 II. ササ群落の生産構造の季節的变化と年純生産量 (英文) 280
- 大島康行: ササ群落の生態学的研究 III. チシマザサの光合成と呼吸 (英文) 349
- 大島康行: ササ群落の生態学的研究 IV. チシマザサ群落の物質生産 (英文) 349
- 大隅正子: 電子顕微鏡による有色体の研究 I. ミカンの有色体の電子顕微鏡的構造 (英文) 165
- 勝見允行: 1:4-ジヒドロナフトエ酸-(I) の作用型に関する研究 I. (英文) 436
- 加藤勇夫: 植物における phosphorylase 作用の研究 I. 気孔発生過程の表皮組織における phosphorylase 作用 (英文) 297
- 菊池正彦: *Penicillium islandicum* Sopp., NRRL 1036 の培養中における菌体色素群の消長 42
- 菊池正彦・中原正城: *Penicillium isolondicum* Sopp. の紫外線照射株における色素生産の消長 463
- 木下祝郎 (→板垣史郎)
- 木村和義: 全暗黒条件下におけるダイコンの花芽分化におよぼす温度および養分の影響 (英文) 361
- 木村勲二・藤生みさ子: ウシグソヒトヨにおける菌傘の開閉性変異 259
- 栗田子郎: シダ類数種の染色体数 (II) (英文) 395
- 桑山弥寿男・庄 貞行・宇佐美正一郎: ミズバショウ花穂の呼吸にみられる青酸促進について 375
- 河野 清・辻井理貴雄・畠山伊佐男: クワの芽の浸透価, 含水量および溶質化の季節的变化 (英文) 118
- 木幡 守 (→板垣史郎)
- 小林 弘: 溪流の底生藻類群落のクロロフィル量について (英文) 228
- 小林 弘: 溪流の底生藻類群落の生産量について (英文) 331

小山鐵夫: ホンモンジスゲ・ゴンゲンスゲ群の変異について (カヤツリグサ科の分類学的研究 13) (英文)	321
斎藤真太郎 (→西田雄行)	
佐伯敏郎: 葉の生長におよぼす物質生産の影響 (英文)	70
佐伯敏郎: 植物群落における葉層の発達についての解析的研究 (英文)	342
堺 恵美 (→柴田万年)	
佐藤公行 (→藤茂 宏)	
柴田万年・堺 恵美: チューリップの2品種のアントシアニンについて (英文)	186
庄 貞行 (→桑山弥寿男)	
杉浦昌弘: マメ種子胚の呼吸の研究 (英文)	19
鈴木静夫: 中沼の湖底泥における水生菌類の季節的变化	30
鈴木静夫・二村坦孝: 水棲不完全菌類の分布と湖沼型 (英文)	51
鈴木静夫: 水生菌類の遊走子の日周的な垂直移動	138
鈴木静夫: 湖水の循環期および停滞期における水生菌類の遊走子の垂直分布	254
鈴木静夫: 酸栄養期における水生菌類の分布と生態	317
鈴木静夫: 湖沼細菌と湖沼型の関係についての培養実験 (英文)	
高見 亘: 花粉の生理学的研究 主として発芽におよぼす微量元素の影響	142
武久 慎: 二倍体ペチュニアの葉の分裂細胞における Aneusomaty (英文)	
建部民雄: エゾミソハギの受精に関する生理学的研究 I. 子房内に含まれる花粉発芽抑制物質 (英文)	291
竹村英一: ヒガンバナ属の人工雑種の形態学的・細胞学的研究 I.	
辻井理貴雄 (→河野 清)	
坪 由宏: 培地のイオン平衡により影響されるクラミドモナスの生殖について (英文)	
坪 由宏: <i>Chlamydomonas moewusii</i> var. <i>rotunda</i> の暗所接合におよぼす照明培養上ずみ液の影響	
寺崎弥助: 新種 <i>Spirillum putridiconchylium</i> について (英文)	79
寺崎弥助: <i>Spirillum</i> 二新種について (英文)	220
豊田清彦: 成熟中のハスの果実の幼芽におけるアスコルビン酸含有量の変化 (英文)	28
仲尾澄子: 細菌の酸化におよぼす低温の影響 (英文)	60
中沢信午: フークス科藻類の発生力学 XVII. 超遠心したスギモク卵における皮部細胞質の差次破壊 (英文)	1
中沢信午: フークス科藻類の発生力学 XVIII. 形成しつつある仮根における原形質要素の配分 (英文)	161
中沢信午: フークス科藻類の発生力学 XIX. スギモク卵の中に極性の細胞骨格がないこと (英文)	
中原正成 (→菊池正彦)	
中村和郎: アカバソカビの胞子の色の突然変異株 (英文)	104
西上一義: 酵母の生長と呼吸におよぼす 2,4-D の影響 (英文)	109
西田雄行・斎藤真太郎: 蕈類胞子の発芽研究 II. イクビゴケおよびハリミズゴケ (英文)	91
西林長朗・猪野俊平: ツルモの遊走子形成 I.	195
二村坦孝 (→鈴木静夫)	
野本宣夫・岩城英夫・門司正三: 耐陰性の生理学・生態学的解析 I. 種々の強さの光のもとでのヤエナリの生長 (英文)	386
橋本 徹: エンドウの茎の1次肥大生長における インドール酢酸とカイネチンの相互作用 (英文)	110
畠山伊佐男 (→河野 清)	
塙 順: ゴマにおける葉の背腹性に関する実験	303
塙 順: ゴマの双生葉形成に関する研究・続報 (英文)	402
肥田美知子・井田和子: メタセコイアの緑葉および紅葉中のカロチノイドについて	369
肥田美智子: 球果の発育からみたスギ科の類縁関係 (追補) メタセコイアの球果の発育	449
肥田美智子: メタセコイアの樹皮、特に巨大コルク細胞について	
広本一由: 子実層分離法による帽菌類の菌糸の培養	134
広本一由: 交配からみたシメジの分類学的考察	419

藤伊 正・石川茂雄: オオマツヨイグサ種子の発芽に対する光照射後の温度の影響について (英文)	414
藤伊 正 (→石川茂雄)	
藤井雅二 (→堀 武義)	
藤生みさ子 (→木村劼二)	
藤茂 宏・佐藤公行: 光化学的亜硝酸還元酵素 (第1報) ホウレンソウから抽出した酵素の 純化およびその性質 (英文)	178
VENKATARAMAN, G. S. (→MEHTA, S. C.)	
堀 武義・藤井雅二: マツバボタンの開花・閉花前後の諸器官における生長素の消長 (英文) ..	357
増淵法之: ナス科植物の接木研究 I. トマト・イヌホオズキのキメラ形成について	34
MEHTA, S. C., and VENKATARAMAN, G. S.: ケイソウのからの微細構造 (英文)	248
村上 浩: アサガオの種子の成熟および発芽過程におけるジベレリンの消長 (英文)	241
門司正三 (→野本宣夫)	
柳下 登: トウガラシの接木雑種に関する研究 I. 接木による果形の変異と第1および第 2の自家受精世代におよぼす影響 (英文)	122
柳下 登: トウガラシの接木雑種に関する研究 II. 反復接木による果形の変異 (英文)	
山本光男: 浸水による豆の割れ目について (英文)	273
横浜康継 (→石川茂雄)	
渡辺光太郎: 穀類花粉の発芽に関する研究 II. 花粉および柱頭の熟度と発芽との関係 (英文) ..	131
和田清美: 無菌培養における春まき小麦の花芽形成について I. 明期の効果と感光期 (英文) ..	236
速 報	
豊国秀夫: 新属サンブクリンドウ属 (仏文)	198
中村輝子・石井愼義・八巻敏雄: マカラスムギの幼葉鞘の細胞内におけるオーキシンの分布 について (英文)	
平畑 規・藤田一夫: カラタチの種子タンパク質の構成アミノ酸について	313
村上 浩: 植物組織におけるジベレリン A ₃ グルコンドの生成	424
雑 録	
井上 浩・北川尙史: <i>Eremonotus</i> 属の日本における初発見	266
浦山隆司: シイタケの三角状楔形竹製の種駒について (英文)	48
SAMPATH, S.: イネ属の分類学的知見	269
豊田清修: 種子中のクロロフィルについての補遺	310
原 襄: 生長点の上からの観察	267
抄 録	86, 315, 427
書 評	86, 426
本会記事	50, 88, 160, 271, 316, 428,

日本植物学会会則

(昭和 35 年 11 月 3 日改正)

第 1 条 本会は日本植物学会という。

第 2 条 本会は植物学の進歩と普及をはかり、あわせて会員おたがいのしたしみを増すのを目的とする。

第 3 条 本会は前条の目的を達するために「植物学雑誌」そのほかの出版物の刊行、大会・講演会・講習会などの開催、そのほか必要と思われる事業を行なう。

第 4 条 本会の会員は次の 5 種とする：

通常会員・終身会員・特別会員・外国通信会員・名誉会員

第 5 条 通常会員とは所定の会費を納めるものを行い、終身会員とは所定の終身会費を納めたものをいう。

第 6 条 特別会員とは引続き本会の会員であつて本会に対していちじるしい功勞のあつた者、外国通信会員とは本会に関係の深い外国人、また名誉会員とは植物学に対して功勞のいちじるしい者で、いづれも評議員会で協議し会長が総会で推薦し承認された者をいう。但しやむを得ない場合は、あとで総会の承認を求めることがある。これらの会員は会費を要しない。

第 7 条 本会には地方支部を置き、会員はいずれかの地方支部に属するものとする。地方支部に

ついでの規定は別に設ける。

第 8 条 本会には次の役員を置く：

会長 1 名・幹事長 1 名・幹事 若干名・評議員 若干名・編集委員 若干名

第 9 条 役員は会員の中から選出し、任期は 2 カ年とする。但し重任することができる。選出についての規定は別に設ける。

第 10 条 会長は会務の全体をすべる。幹事長は会長を助けて会務を処理する。幹事は庶務・会計・編集・図書管理など日常の会務を行なう。

第 11 条 評議員は評議員会を構成する。評議員会は会長の諮問の範囲で本会の要務を審議し、また総会への提案を作る。

第 12 条 編集委員は編集委員会を構成する。幹事長はその長となる。編集委員会は「植物学雑誌」の編集に関しての一切の責任を負う。

第 13 条 本会の会計年度は 1 月に始まり 12 月に終る。

第 14 条 本会は原則として毎年 1 回総会を開き、会務を協議し議決する。なお会長が必要と認めた場合には臨時総会を開くことができる。

第 15 条 本会は総会の時大会を開き研究発表などを行なう。大会には大会会長そのほか若干名の(裏面へつづく)

きりとり線

入会申込書

氏名	男 女	この紙を切りとつて所要の事がらを記入または○でかこみ会費をそえて学会あてにお送り下さい。どなたでも入会できます。		
ふりがな	明治 大正 昭和 年 月 日生			
勤務先 (所在地)				
住 所				
通常 終身	会員に	昭和 年 から	支部へ	雑誌の送り先を指定して下↑ さい。希望する方へ○印を

入会の申込、会費(年 1200 円) 予約購読(年 2000 円)の払込、そのほか会へのご連絡のあて先は：

東京都文京区東京大学理学部植物学教室 日本植物学会です。

それから会費の払込は振替貯金口座東京 11190 番を利用されるのが最も確実です。なお振替でお払込の場合は特に領収書をさし上げませんからあしからず。

臨時の役員を置くことができる。大会会長は会長が推薦し、そのほかの役員は大会会長が依頼する。

第16条 会員は会合に出席して講演をし 議事に参加し、「植物学雑誌」に投稿し、また本会所有の図書を観覧することができる。また毎号無料で「植物学雑誌」の配布を受ける。

第17条 会員が退会しようとするときは、そのことを本会に通知しなければならない。もし会費

の滞納があるときはその際全額を納めなければならない。但し既に納めた会費は一切これを返さない。通常会員が会費を滞納したときは直ちに前条の権利を停止し、1 年以上滞納したときは除名することがある。

第18条 本会の会則または 付則を変更するには総会または臨時総会でこれを協議し、出席会員の3分の2以上の同意を得なければならない。

付則第 1 会 費 (会則第 5 条関係)

第 1 条 通常会員の会費は年 1200 円とし 400 円ずつ分納することもできる。終身会費は 20,000 円とする。

このほか国外在住会員に限り植物学雑誌の送

料を負担する。

第 2 条 評議員編集委員以外の役員は在任中会費を要しない。

付則第 2 地方支部 (会則第 7 条関係)

第 1 条 地方支部は原則として 50 名以上の会員のある地方に置き、北海道・東北・関東・北陸・中部・近畿・中国四国・九州の 8 とする。

第 2 条 会員は居住地の支部に入るのが原則であるが、事情により他の支部に属することもでき

る。

第 3 条 支部には支部長を置く。支部長は支部を代表する。

第 4 条 そのほかの規定は各支部ごとに設ける。

付則第 3 役員の選出方法 (会則第 9 条関係)

第 1 条 会長は全会員の投票で選出される。その際評議員会は若干名の候補者を推薦することができる。

第 2 条 評議員は各支部別に支部会員によつて選出される。その定員は各支部最低 2 名とし、会員数が 100 名を越える支部では 50 名までごと

に 1 名を増す。評議員は引続き 3 期選出されることはできない。なお会長および幹事長は評議員を兼任することができない。

第 3 条 幹事長・幹事・編集委員はいずれも会長が依頼する。

きりとり線

入会や転居などの場合には必ず別に支部へも連絡して下さい。支部のあて先は次のとおりです。なおどの支部へ入るかは大体地理的にきまるわけですが、ご都合で A 支部よりも B 支部の方が便利だという方は B 支部に入られてもよいことになっています。

北海道支部 札幌市北 8 条西 5 丁目 北海道大学理学部植物学教室

東北支部 仙台市片平丁 東北大学理学部生物学教室

関東支部 和浦市常盤町 埼玉大学文理学部生物学教室

北陸支部 金沢市仙石町 金沢大学理学部植物学教室

中部支部 名古屋市千種区不老町 名古屋大学理学部生物学教室

近畿支部 京都市左京区北白川 京都大学理学部植物学教室

中国四国支部 広島市東千田町 広島大学理学部植物学教室

九州支部 福岡市箱崎 九州大学理学部生物学教室

Developmental Mechanics of Fucaceous Algae XVII. Differential Destruction of the Cortical Layer of Cytoplasm in Ultracentrifuged *Coccophora* Eggs

by Singo NAKAZAWA*

Received May 14, 1960

In some fucoids such as *Fucus serratus*¹⁾, *Coccophora Langsdorffii*²⁻⁵⁾, *Sargassum confusum*⁵⁾ and *S. tortile*²⁾, their developmental axis cannot be determined even if the intracellular materials of the egg are stratified by means of centrifuging. This indicates that the factor which controls the polarity of these fucoid eggs is not the arrangement of the internal protoplasmic components but it consists in a certain differentiation of the cortical layer of the cytoplasm or of the plasma membrane which is immovable by centrifuging. A similar idea has been presented for the polarity of animal eggs by many authors⁶⁾. On fucoid eggs, it was suggested by Nakazawa²⁾ and was advocated by Bünning⁹⁾ and Jaffe¹²⁾. However, it is still questionable whether or not the cortical layer including the plasma membrane is absolutely immovable by the centrifugal force. Herein, the present experiment was undertaken. It also aims at confirming what are the kinds of strata brought about by centrifuging, because it has not always been ascertained yet so far as in *Coccophora* eggs.

Material and Method

Coccophora Langsdorffii was collected in Asamushi, Aomori Prefecture, and was cultured in glass vessels with filtered sea water. After egg liberation, the eggs were fertilized artificially. Just after fertilization, the eggs were ultracentrifuged at 25,000 times gravity for five minutes by use of an air-turbine centrifuge. The stratification was inspected by use of a microscope. The normal and the centrifuged eggs were immersed in hypotonic sea water at various concentrations for observing the plasmolysis pattern. They were also immersed in starfish toxin dissolved in normal sea water at a variety of concentrations in the expectation that differential destruction of the cortical layer of the cytoplasm would take place under the effect of the toxin as was preliminarily reported before¹⁰⁾. The toxic substance was obtained by extraction from the stomach of *Asterias amurensis* collected in Asamushi as follows: (1) the stomach was homogenized, (2) to which was added five to ten times in volume of pure methanol, (3) left for 24 hours for extraction, (4) filtered, (5) the filtrate was distilled, (6) the remnants were dried over a water bath and were preserved in a desiccator at room temperature. In the experiment, the toxin thus obtained was dissolved in normal sea water. The solution was of pH 7.4 at 10^{-8} in concentration, pH 7.5 at 5×10^{-4} , and pH 8.2 at or below 10^{-4} which was equal to the pH of normal sea water. Therefore, the effect of starfish toxin cannot be attributed to the change in pH of the sea water.

* Biology Department, Yamagata University, Yamagata, Japan.

Results with Discussion

The intracellular materials were clearly stratified at least into five layers. At the centripetal end, the so-called oil cap appears displaying yellow color. The oil cap is liable to be thrown out of the egg cell by centrifugal force into the space intervening between the egg cell and the surrounding coat of mucilage (Fig. 1B). Next to this, there comes a transparent layer which was presumed by Levring¹¹⁾ to be composed of water. The third layer is composed of brown plastids and the nucleus. The fourth is a colorless layer presumed to be consisting of hyaline cytoplasm. In the fresh material, the fourth layer appears to be extending to the centrifugal end. Immersed in distilled water, the fifth layer becomes distinguishable from the fourth exhibiting dark grey granules occupying the centrifugal half of the egg cell (Fig. 1C).

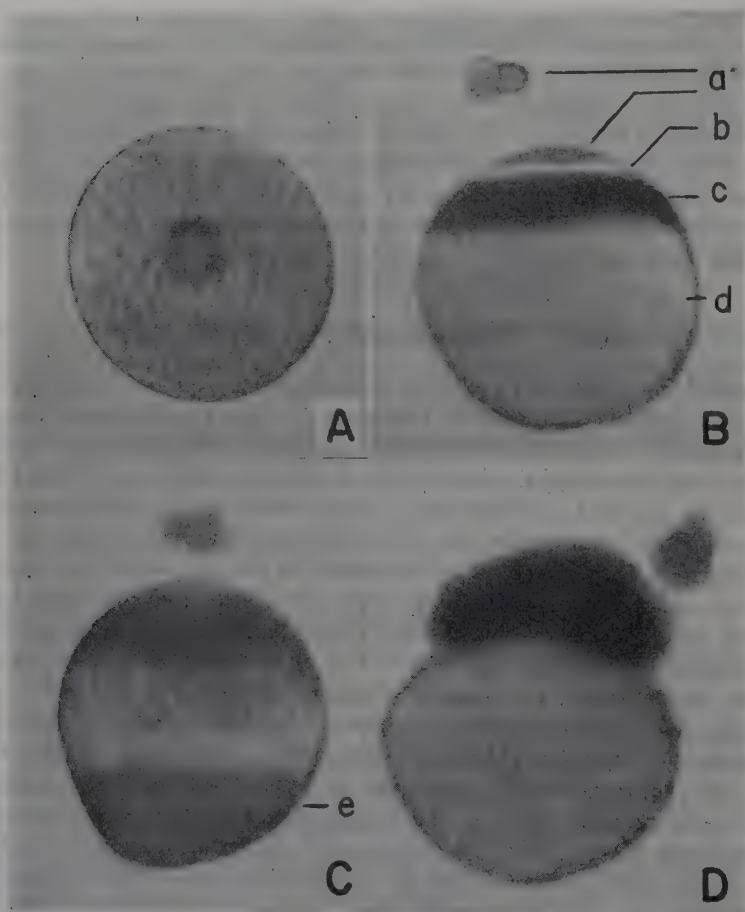


Fig. 1. A) normal egg of *Coccophora* just after fertilization, B) the same centrifuged, C) darkening of the layer of granules with distilled water, D) destruction of the membrane in a subcentripetal zone affected by starfish toxin. a, oil cap; b, free water layer; c, plastids and nucleus layer; d, hyaline cytoplasm layer; e, granule layer.

When the normal egg is immersed in distilled water or in hypotonic sea water, the cell is expanded by absorbing water and plasmoptysis occurs within ten minutes or so. On this occasion, the site where the plasma membrane is ruptured is not definite. The relation between the occurrence of plasmoptysis and sea water concentration is indicated in Table 1. Prior to the occurrence of the plasmoptysis, a number of dark grey granules appear in the subcortical part of the cytoplasm and then a part of the plasma membrane is suddenly ruptured, and the dark granules jet out of the cell with other elements of the cytoplasm. But, when the centrifuged egg is immersed in distilled water, the site of plasmoptysis appears usually in the centrifugal half where the dark granules are stratified.

When the normal egg is placed in the solution of the starfish toxin at a suitable concentration, the plasma membrane is destroyed. The relation between the concentration of the toxin and the destruction is shown in Table 2. In the egg before or

Table 1. Occurrence (+), non-occurrence (-), and the intermediate occurrence (±) of plasmoptysis at various concentrations of sea water.

Sea water concentration %	Plasmoptysis
100	—
60	—
50	±
30	+
20	+
Distilled water	+

Table 2. Destruction (+), non-destruction (-), and the intermediate (±) of the plasma membrane at various concentrations of the starfish toxin.

Concentration	Destruction
10^{-8}	+
5×10^{-4}	+
10^{-4}	+
5×10^{-5}	±
10^{-5}	—
Control	—

just after fertilization, the destruction appears uniformly all over the cell and the cytoplasm is scattered. But in the egg which was transformed to an ovate form i.e. after differentiation of the rhizoid pole, it tends to take place at or near the rhizoid pole. When the centrifuged egg is immersed in the toxin solution, the destruction occurs always in a subcentripetal zone covering the fourth layer, that is, at the part a little centrifugal from the plastid layer with the result that the centripetal three layers including the plastids and nucleus are separated from the rest.

Centrifugation reveals that the protoplasm of *Coccophora* eggs is composed of mainly six kinds of materials: oil drops, free water, plastids, nucleus, hyaline cytoplasm, and granules darkened with hydration. The last granules are equal to those formerly described as stainable with Bismarck brown Y³). Distinction of the layer of hyaline cytoplasm from the granules is a new information confirmed by the present experiments. It is noteworthy that both plasmoptysis and destruction of the cortical layer of cytoplasm including the plasma membrane take place differentially in the centrifuged egg, though these occur uniformly in the normal egg. This indicates that the centrifuging brought about a certain polar differentiation in the nature of the cortical layer. Prior to the occurrence of plasmoptysis in the centrifuged egg, the most centrifugal layer is darkened by distilled water, and this darkening usually appears first at or near the centrifugal end, then spreads towards the centripetal side. Therefore, it seems that water permeability is highest in the centrifugal part. The darkening is attributed to the change in color by hydration of granules stratified

centrifugally. Therefore, the darkening never spreads to the hyaline stratum. In normal eggs, the same granules are found in subcortical zones of the endoplasm. The destruction of the plasma membrane by the starfish toxin occurs uniformly in the normal egg as was reported by Nakazawa¹⁰). But, in the centrifuged egg, it occurs selectively on the part corresponding to the layer of the hyaline cytoplasm, and the more centripetal part of the egg is separated from the rest (Fig. 1D). On this occasion, at first each layer stratified does not seem to be destroyed. Therefore, the destruction seems to be restricted at least primarily to the cortex of cytoplasm. Afterwards, however, all the ingredients of the protoplasm is destroyed so that they collapse and scatter.

Thus, the ultracentrifuging results in the differentiation of the plasma membrane, or the cortical layer of cytoplasm, at least into two different zones. One is the appearance of the zone characterized by the highest permeability to water at the centrifugal end. The other is the determination of a subcentripetal zone which is especially susceptible to the starfish toxin. Nevertheless, it is certain that even the same or the stronger centrifuging is ineffective upon the determination of the polarity axis^{4,5}). This strongly implies that though the polarity axis is presumed to be determined fundamentally by a differentiation of the plasma membrane or the cortical layer, it must be differentiation of another kind not like those brought about in the present experiments of ultracentrifuging. As to the polarity determination by centrifuging in *Cystoseira*¹³) and in *Fucus*^{6,7}), no remark is permitted from the present experiments.

Summary

Eggs of *Coccophora Langsdorffii* was ultracentrifuged just after fertilization at 25,000 times gravity for five minutes. As a result, the following was discovered.

(1) The intracellular materials are stratified into at least five layers. Arranged in order from the centripetal end, they are: the oil cap, the transparent layer of free water, the plastids and nucleus layer, the hyaline cytoplasm layer, and the granules layer.

(2) When the centrifuged egg is immersed in distilled water, or in hypotonic sea water below 30 per cent in concentration, the granules stratified in the centrifugal zone are darkened in color and the darkening begins at or near the centrifugal end and then spreads towards the centripetal side, then the plasmoptysis occurs in the centrifugal half. This indicates that the water permeability is highest at the centrifugal end.

(3) When the centrifuged egg is immersed in the solution of the starfish toxin at or over 10^{-4} in concentration, the plasma membrane is destroyed in the subcentripetal zone surrounding the layer of hyaline cytoplasm. This indicates that a certain differentiation in susceptibility to starfish toxin has taken place in the plasma membrane or the cortical layer with the centrifugation. That is, the cortical layer including the plasma membrane is also movable by centrifuging, though the movement is invalid in determining the polarity axis.

The writer expresses his cordial thanks to the late Prof. Eishiro Sawano for his preparation of the starfish toxin.

References

- 1) Beams, H. W., J. Mar. Biol. Ass. United Kingdom **21**: 571 (1937). 2) Nakazawa, S., Sci. Rep. Tohoku Univ. 4th Ser. **19**: 73 (1951). 3) —, Bot. Mag. Tokyo **70**: 1 (1957). 4) —, Sci. Rep. Tohoku Univ. 4th Ser. **23**: 119 (1957). 5) —, Naturwiss. **46**: 333 (1957). 6) Whitaker, D. M., Biol. Bull. **73**: 249 (1937). 7) —, J. Cell. Comp. Physiol. **15**: 173 (1940). 8) Motomura, I., Sci. Rep. Tohoku Univ. 4th Ser. **18**: 117 (1949). 9) Büning, E., Protoplasmatologia **8**, **9a**: 1 (1957). 10) Nakazawa, S., Bull. Jap. Soc. Phycol. **4**: 52 (1956). 11) Levring, T., Physiol. Plantarum **5**: 528 (1952). 12) Jaffe, L., Exp. Cell. Res. **15**: 282 (1952). 13) Knapp, E., Planta **14**: 731 (1931).

摘 要

中沢信午： フークス科藻類の発生力学 XVII. 超遠心したスギモク卵における皮部細胞質の差次破壊

スギモク (*Cocophora Langsdorffii*) の卵を受精直後に重力の 25,000 倍で 5 分間超遠心した結果、つぎのことが知られた。

(1) 卵内容は求心端から油滴、水、プラスチドと核、透明質、および細粒子の順に層にわかれる。

(2) 遠心した卵を蒸留水または 30% 海水以下の低張液にいれると、遠心端の細粒子は暗色になる。この暗色化は遠心端にはじまり、しだいに求心側にむかってひろがり、やがて遠心側で皮部細胞質層が破れ、原形質吐出がおこる。これは遠心端で水の透過性をもっとも高いことを示している。

(3) 遠心した卵をヒトデ *Asterias amurensis* から抽出した毒素 10^{-4} 以上をふくむ海水にいれると求心端からやや遠ざかったところから原形質膜が破壊する。これは原形質膜または皮部細胞質層がこの領域で特に感受性が高く分化していることを示す。つまり皮部細胞質層は遠心力でうごかされ得る。しかしうごかされた結果、それが極性軸を決定することはできない。(山形大学生物学教室)

On the Spatial Difference of the Primary Production in the Lake and its Relation to Environmental Factors

by Shun-ei ICHIMURA*

Received June 10, 1960

Regarding the horizontal distribution of phytoplankton in the lake, the noticeable information has been provided by many investigators. The features of the horizontal distribution should be formed by the interplay of the physical actions, such as wind, in- and outflowing streams and contour shape of lake basin, and the spatial difference in growth rate of phytoplankton in the lake. However, so far almost all of the illustrations for the causes which determine the horizontal distribution have been made only through the physical actions and they have slightly been touched from the ecological viewpoint.

The spatial difference of the productivity must precisely be examined in relation to the study of the primary production in the lake, but the data in this field are still inadequate as to Japanese lakes.

Concerning the above subjects, therefore, the present study has been carried out in several lakes with different morphometries, and the features of the horizontal distribution of phytoplankton were pursued on the basis of the dry matter production.

Types of horizontal distribution of phytoplankton in the lake

The standing crop of phytoplankton was measured in chlorophyll amount. Fig. 1 shows the representative features in the distribution of chlorophyll in a vertical section from the top to the end of lakes and the spatial difference in the mean value of

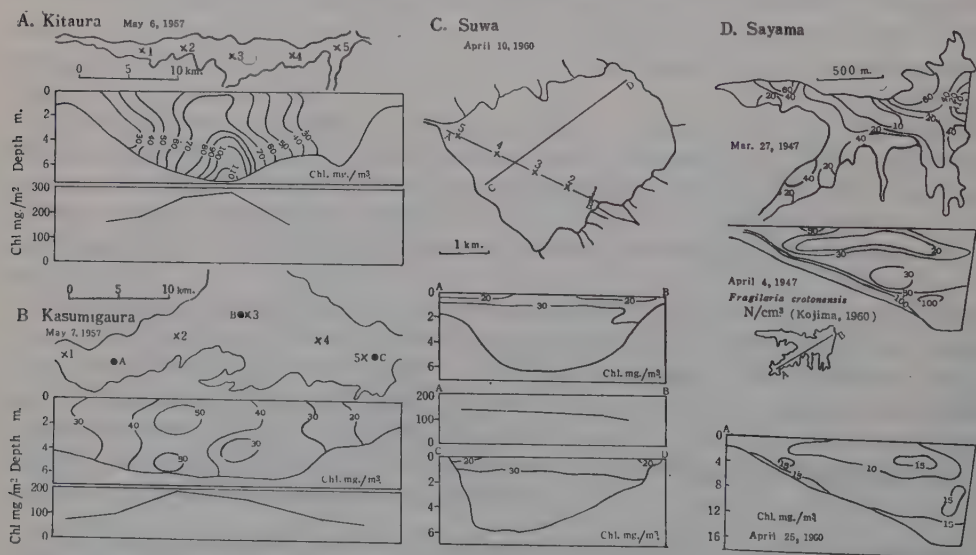


Fig. 1. Distribution of chlorophyll in lakes.

* Botanical Institute, Faculty of Science, Tokyo University of Education, Otsuka, Tokyo, Japan.

the chlorophyll amount in euphotic layer. The patterns of the horizontal and vertical distributions differed from one another according to the contour shapes of lakes and they were classified into three types, the uniform, regular and irregular ones.

The uniform distribution type is found usually in the oval-shaped lakes with simple shore line and uniform depth such as Lake Suwa (Nagano Prefecture), in which the effect of the inflowing water is insignificant. The phytoplankters in these lakes scatter homogeneously in all part of the lake.

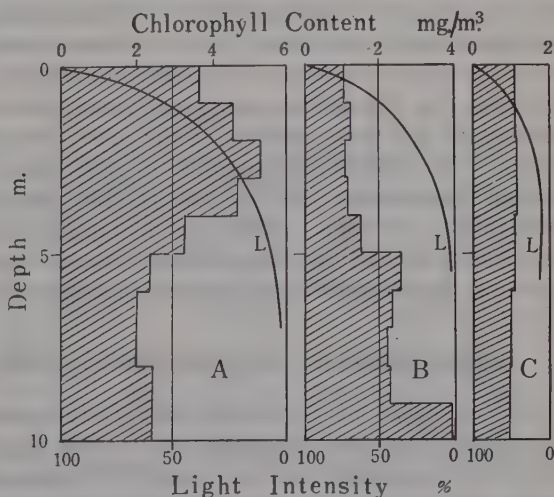
The regular distribution type is common to the slender lakes such as Lake Kitaura (Ibaraki Prefecture) with simple shore line. As for this type, the standing crop measured in the upper region, especially in the area surrounding the mouth of the inflow, is relatively small in comparison with the middle part, where the standing crop is generally higher, but it decreases successively from the halfway region to the lower end of the lake.

The irregular distribution is obtained in the lakes with a complicated shore line and also in the reservoirs, in which some part of the water is exchanged continuously through the inflow and discharge. In this pattern, the standing crop of phytoplankton is more abundant in the lentic environments than in that of the lotic environments. But the irregular distribution pattern is only of conditional character.

Spatial difference in productive structure of phytoplankton community in the lake

According to recent theoretical analyses performed by some investigators¹⁻³), the pattern of the vertical distribution of phytoplankton can be summarized as being the two general types following; the one is the homogeneous type and the other is the

Fig. 2. Productive structure of phytoplankton communities. A: Stratum productive structure, Lake Yamanaka, Sept. 12, 1951. B: L-shaped productive structure, Aug. 25, 1951. C: Homogeneous productive structure, Lake Nakatsuna, July 14, 1953.



stratum one (cf. Fig. 2). The homogeneous type is usually established during the circulation period, while the stratum one, during the stagnation period. Among the stratum types, L-shaped pattern stratifies slightly in the euphotic layer but chlorophyll is extremely abundant in the destrophic layer, in which photosynthesis is normally not performed. This L-shaped pattern has usually been found in the deep lakes during the stagnation period. The foregoing two distribution types have been ascertained practically *in situ*⁴) and it has also been confirmed that these types appear

alternately in the course of the year in the lake. However, the productive structure differs spatially in one and the same lake. Such variation was observed remarkably during the stagnation period in the deep, slender lakes. As an illustration, the results obtained in Lake Kitaura are indicated in Fig. 3-A. The productive structure observed

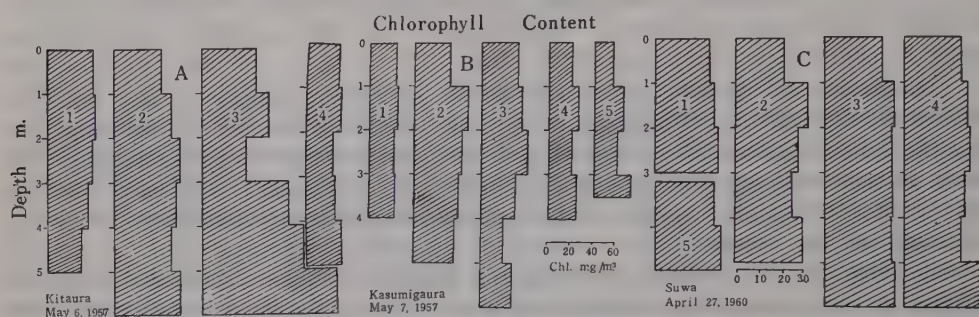


Fig. 3. Spatial difference of productive structure of phytoplankton community in lakes.

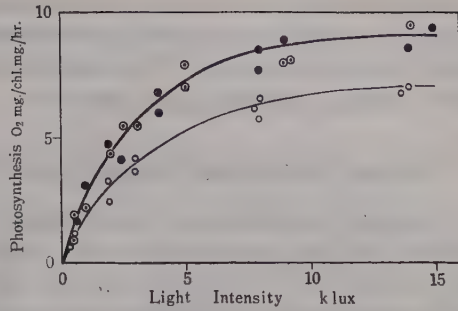
at the location near the top of the lake was of the homogeneous type and the same type could be found everywhere in the littoral regions. With the increasing distance from the mouth of the inflow, the homogeneous type transformed gradually into the stratiform type which was converted further into the L-shaped type at the middle of the lake. In the lower shallow region of the lake, the L-shaped type transferred again into the homogeneous one.

In the shallow lake as well as in the round-shaped deep lake with uniform depth there is no spatial difference in the pattern of productive structure, but the structure is usually represented by the homogeneous type in the former and by the stratiform one in the latter. Fig. 3-B and -C show the productive structures obtained from the various stations of Lakes Kasumigaura and Suwa. The areas of these lakes are large but the depths are rather small in proportion to the volumes of the lakes. The upper and lower regions of the lakes are 2-3 m. in depth, and the middle regions, 6-7 m. The productive structures in the slender Lake Kasumigaura were found to be the homogeneous type in both its upper and lower regions and the slightly stratiform one in the deep, middle region. In the round-shaped Lake Suwa, the productive structure indicated the homogeneous one at every place. The mode of the stratification may be determined by the morphometric difference of the basin.

Spatial difference of photosynthetic activity of phytoplankton in the lake

As reported in a previous paper⁵), the photosynthetic activity of phytoplankton in the ocean differed regionally but it was approximately the same in a restricted area. As compared with the area of the ocean, that of a lake is small in a scale, but here the photosynthetic activity of phytoplankton community differs place to place even within a lake. In a small lake, such activity difference is rather slight but it appears clearly in a large lake. Fig. 4 shows the photosynthesis-light curve obtained under the laboratory condition in the sample waters taken from the surface of three stations of Lake Kasumigaura. The photosynthesis was measured by the Winkler method. Sample A was taken at the upper region, sample B at the middle and sample C at the lower region, and the distance between the two stations is about 10 km. The Samples A and B were entirely the same in the light-photosynthesis reaction

Fig. 4. Photosynthesis-light curves measured in sample waters from upper, middle and lower regions of Lake Kasumigaura on September 17, 1956. \bullet —: station A, \odot —: station B, \circ —: station C

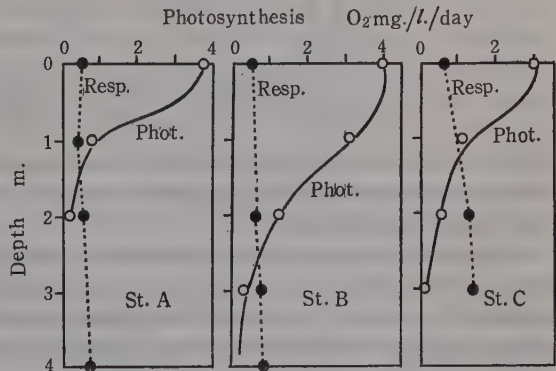


as well as in the maximum rate, and were a little higher than that of sample C.

The successive decrease in photosynthetic rate with the downward stream flow was measured more clearly during the stagnation period in long slender lakes with complex shore lines. For example, the photosynthetic rate measured in Lake Jonuma in September 11, 1957 was 14–17 CO₂ mg./chl.mg./hr. in the sample taken at the region near the mouth of inflow, but only 6–8 CO₂ mg./chl.mg./hr. at the lower region of the lake. As will be illustrated in the next chapter, these variations in photosynthetic rate are referred to the varying environmental conditions, especially nutrient gradient.

As pointed out in a previous paper⁶⁾, the photosynthesis pattern of natural phytoplankton concerning light intensity varies with the difference of its habitat: the phytoplankters in the surface layer are the sun form, and the samples in deep layer, the shade form. Such differentiation of photosynthesis pattern, however, was found only in the samples taken vertically from the stations with deep basins and no differentiation was observed in the samples from the shallow regions. Therefore, it can be deduced that the degree of the differentiation alters according to the difference of the depth at each region in a lake.

Fig. 5. Depth-photosynthesis curves from stations 1, 2 and 3 in Lake Kasumigaura on September 23, 1957.



The features of the vertical change in the photosynthetic rate *in situ* also varied with the difference of the stations. Fig. 5 indicates the depth-photosynthesis curves measured *in situ* at the said three stations in Lake Kasumigaura. The Secchi disc reading at that time was 1.7 m. at station B, 0.7 m. at station A, and 0.9 m. at station C. The photosynthetic rate decreased rapidly with the increasing of the depth both at stations A and C, and the compensation depth was found at about 1.2–1.5 m. The vertical change in the photosynthetic rate occurred more slowly at station B than at stations A and C, and the compensation depth was 2.5 m.

For these reasons, the unit areal production at the middle region may be surmised to be more large value than that obtained at the shallow regions of the upper and lower regions of the lake. The same phenomenon had also been observed by Steemann Nielsen⁷⁾ in Lake Fure, Denmark.

Factors determining horizontal distribution of phytoplankton in the lake

Since the phytoplankters are scattered in the water, the horizontal distribution of phytoplankton may correspond mainly to the features of the water current and it may also result from the difference in the production rate of phytoplankton due to the spatial difference of the environmental conditions in the lake.

Simple relationship between the horizontal distribution and the environmental factors can be found in a shallow lake with simple shore line and flat basin. The entire water in the shallow lake is agitated completely by the natural actions of wind and water current, thereby the environmental conditions in such a lake being continuously in a uniform state and the phytoplankton is scattered homogeneously all over the lake. Because of the uniform condition in the lake, the photosynthetic activity of the phytoplankton is equivalent everywhere and the spatial difference of the unit areal primary production is little worth considering, consequently the standing crop would always maintain the homogeneous distribution pattern.

The uniform type was found also in a small, deep lake with round contour shape, in which the environmental conditions are horizontally the same. The circulation of the water in such lakes occurs only in the upper layer and it is relatively gentle, thereby the breakdown of the productive structure being slight and progressing slow-

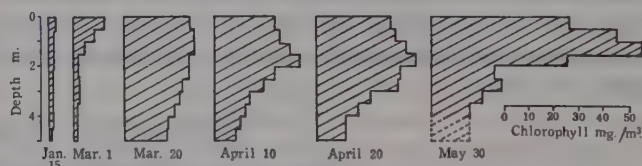


Fig. 6. Seasonal change of productive structure of phytoplankton community in Lake Nakanuma.

ly. As is well seen in Fig. 6, under these conditions, the developmental process can clearly be observed in the productive structure which indicates an analogous structure constructed theoretically.

The lakes, in which the phytoplankton distributes with regularity, resemble each other in their slender and deep forms. In such lakes, both upper and lower regions are usually shallow, while middle region is much more deeper than the former ones and the regions widely differ from each other in their aquatic environments such as light, nutrient concentration, etc. In the shallow regions, the turbidity of water is very high and through which the penetrating light is usually absorbed rapidly by the water, while the rapidity of light extinction decreases in the middle region with deep water as the result of the low turbidity. On the other hand, the chlorophyll content in the shallow region is small in spite of the high turbidity. This may suggest that the ratio of the non-living matters to the phytoplankton in amount is much larger in the shallow region than in the deep region due to the rising of bottom mud induced by water stirring, the light penetrated into the water being reduced mainly by the non-living matters. As indicated in Fig. 7, the features of the vertical change in

photosynthetic rates, which are calculated for the said three stations in Lake Kasumigaura by means of combining the photosynthesis-light curve in Fig. 4 and the light-depth curve measured *in situ*, coincided fairly well with the results in Fig. 5. The gross primary production calculated by the combination of the depth-photosynthesis curves in Fig. 7 and the chlorophyll amount *in situ* were 2.95 O_2 g./m²/day at station A, 6.25 O_2 g./m²/day at station B and 2.47 O_2 g./m²/day at station C. Therefore, it seems reasonably certain that the unsuitable light condition in the shallow regions may contribute directly to the reduction of the organic matter production per unit surface area in the phytoplankton community there.

Another conceivable reason for the small standing crop in the upper region may be referred to the effect of the inflowing water. Since the upper region is replenished with the inflow stream, the phytoplankton growing there is diluted always with the inflowing water and a large part of the phytoplankton is carried away with the stream from that place to the halfway down region. Hence, the standing crop hardly reaches a maximum equilibrium level at the upper-stream region.

In the middle region of moderate transparency, the light can extend to considerable depth of the water and the compensation depth is much deeper than those both in the upper- and downstream regions. In addition to this suitable light condition, the nutrients in the water have not yet been exhausted in this region (see Fig. 8), thereby the photosynthetic activity of phytoplankton being also high. Besides the facts mentioned above, the velocity of the current is slow in the middle region where the amount of production surpasses the amount which has escaped from there to the downstream region. Under these circumstances, the dry matter production in the middle region can be expected to give high yield as compared with that in the other regions. Because the wave action is rather weak in the middle region of the lake, the vertical shift of phytoplankton is minor and only the precipitation of phytoplankton progresses, and thereby the L-shaped structure can be formed at a region somewhat lower than the halfway down region. Simultaneously, the accumulation of nutrients proceeds in the middle region through the biological production and a large part of the nutrients accumulated in phytoplankton cells is stored in deeper layer in the form of organic deposits, some of which undergo decomposition and consequently the bottom water is enriched. As a result of the accumulation of salts in the deeper layer of the middle region, the lower end of the down stream of the lake is conserved usually with the exhausted water, especially during the summer stagnation period. This phenomenon could be speculated clearly in deep, slender lakes. Fig. 8-A indicates the values of phosphate-P content both in the surface and bottom waters of Lake Kitaura at various stations in the summer stagnation period. The amount of phosphate-P decreased rapidly in the surface water with the gradual downward flow, while its value in the bottom water rather increased at the middle region. According to the data measured in Lake Jonuma, the magnitude of the nutrient gradient

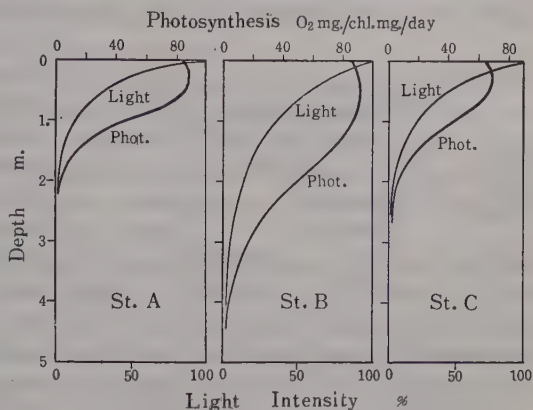


Fig. 7. Vertical change in photosynthetic rate calculated from Fig. 4 and depth-light curve measured *in situ* in Lake Kasumigaura.

depends upon the lake contours and it is extremely great at the growing period. Since the nutrient elements of this lake were mostly exhausted at station 1, the effect of the enriched sewerage did not reach to the narrow, slender part (station 2) of the lake. As illustrated in Fig. 8-B, the highest value was obtained at the mouth

of the inflow of the sewerage and the next was measured at the middle part (stations 4 and 5) and finally nutrients were scanty or practically of a negligible amount at the lower end of the lake, while the gradient was not distinct during the circulation period. Beside the gradient of nutrient concentration, it is interesting to note that the feature of the distribution of the standing crop and the difference of the photosynthetic activity coincides very well with the nutrient gradient. Judging from the fact that the photosynthetic activity in the raw water increases with addition of nutrients⁹), it can be deduced that the reduction of primary production in lower region may be partly caused by the deficiency of the nutrients. As one of the essential factors determining the horizontal distribution of phytoplankton, therefore, some understanding is necessary on the horizontal distribution of the nutrients in the lake.

The mosaic pattern in the horizontal distribution of phytoplankton within a lake is resulted mainly from the partial difference in the flowage. As reported by Kojima⁹) and Stepanek *et al.*¹⁰), the irregular distribu-

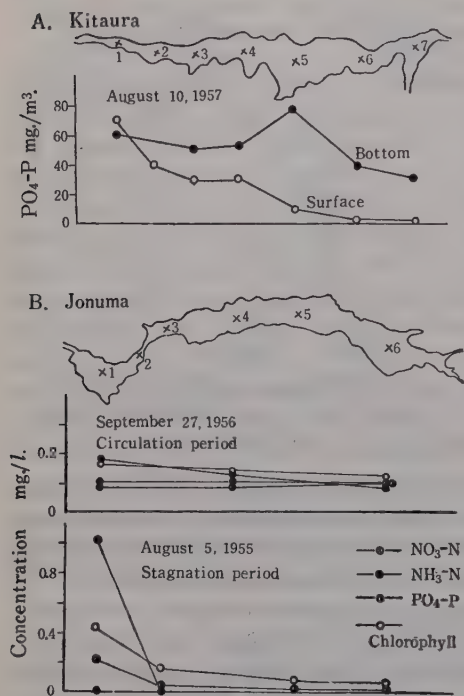


Fig. 8. Nutrient content in waters of Lake Kitaura and Jonuma.

tion is common in the reservoirs, where the water body is divided distinctly into two parts, one is the running water and the other is the dead water. In the former, the water is continuously renewed with the inflow and through which the standing crop of phytoplankton is diluted and moreover it is removed with the discharge. On the contrary, the phytoplankton in the dead water region increases successively within the same region.

Summary

Concerning the difference of the primary production in vertical and horizontal directions in the lake, the present researches were carried out in several lakes which differ from each other in their contours. The main factor determining the features of the distribution pattern was elucidated through the analysis of the interrelationship between the dry matter production by phytoplankton and the environmental conditions.

1) As for the horizontal distribution of the phytoplankton, three types can be observed. The first is the uniform distribution that is found in the shallow lake. The second is the regular one and observed in the slender lake with a simple shore

line, lacking in any large inflows and outflows. The third type is the irregular one and is obtained in the lake and reservoir with a complicated water current.

2) The different productive structures are found in different parts of the lake. The homogeneous structure is found in the shallow region of the lake and the stratified one can be observed in the regions with deep water.

3) The photosynthetic activities of phytoplankton differ spatially in a lake. Generally, the photosynthetic activities of phytoplankton sampled from the upper and middle regions of the lake are entirely the same, but that from the lower region shows usually low activity than those of the samples from the former two regions. Differentiation of phytoplankton to the sun and shade forms is observed in the region with deep basin, but not noticed in the shallow region.

4) The spatial difference in the light and nutrient conditions in a lake has been confirmed as the factors determining the horizontal distribution. Unfavorable light condition of the shallow region, which is caused by high turbidity through the bottom mud shifting, reduces the photosynthesis of phytoplankton, and consequently the dry matter production. The light condition in the region with deep water is suitable for the production.

5) Since the nutrients are exhausted at the upper and middle region of the lake, the lower region is replenished usually with the poor nutrient water. The nutrient gradient in the lake affects remarkably the spatial difference in the productivity of the slender lake with complex shore line.

The author should like to express his sincere thanks to Prof. M. Monsi and Prof. K. Hogetsu for their invaluable advice and encouragement throughout this investigation.

References

- 1) Hogetsu, K., and Ichimura, S., Jap. Jour. Bot. **14**: 280 (1954). 2) Talling, J. F., New Phytologist **56**: 133 (1957). 3) Saeki, T., and Kuroiwa, S., Bot. Mag. Tokyo **72**: 27 (1959). 4) Ichimura, S., *ibid.* **69**: 7 (1956). 5) —, and Saijo, Y., *ibid.* **72**: 193 (1959). 6) Ichimura, S., *ibid.* **73**: 458 (1960). 7) Steemann Nielsen, E., Furesoundersogelser 1950-54, Folia Limnologica Scandinavica **10**: 104 (1958). 8) Ichimura, S., Bot. Mag. Tokyo **71**: 261 (1958). 9) Kojima, S., Suido Kenkyu **40**: 1 (1960). 10) Stepanek, M., and Chalupa, J., Papers from Inst. Chem. Technol., Prague, 1958, Faculty of Technol. of Fuel and Water **2**: 313 (1958).

摘 要

市村俊英：同一湖沼の異なった場所における基礎生産のちがい

同一の湖沼内において、植物プランクトンの場所による基礎生産のちがいを調べた。円形の単純な湖盆形態をもつ湖沼では、著しい相違は見られず、全水域にわたってほぼ均一であった。長形の湖沼では流れとともに現存量、生産力ともに減少する規則的な変化がみられた。複雑な湖盆形態と湖流をもつ湖沼では場所によるちがいは全く不規則であった。本論文ではこのような変化様式が湖盆形態によって規定される環境条件と、これにともなう物質生産の相違によって定まることを明らかにした。(東京教育大学理学部植物学教室)

Photoperiodic Control of the Germination of *Eragrostis* Seeds

by Sigeo ISIKAWA*, Tadashi FUJII* and Yasutsugu YOKOHAMA*

Received July 11, 1960

In studies on seed germination under various conditions, it was found that seeds of several species germinated better under long daily light periods than under short ones^{1,2,3}).

The photoperiodic control of seed germination has been reported in a number of photoblastic seeds by Isikawa⁴). Black and Wareing⁵) demonstrated the requirement of long photoperiods for high germination of *Betula pubescens* Ehr. seeds and its dependence on temperature. In addition they showed that the length of the daily dark period played a more determinative rôle than did the length of the light period in this photoperiodic response, as in the flowering responses in plants.

Isikawa indicated that many of the "Light-favored Seeds" were "Short Day Seeds" the germination of which was promoted in the presence of the dark period like the flowering of short-day plants⁴). Black⁶) and Black and Wareing⁷) have investigated the responses in such a "short-day" seeds, and demonstrated that the response is markedly photoperiodic, a high germination percentage being obtained under short days. A few further theoretical reports, however, have been written concerning the requirement of promotive dark periods for germination. The present studies were undertaken to investigate the requirement for seed germination of the dark period corresponding to that in flowering of short-day plants.

Material and Methods

The work performed with *Eragrostis ferruginea* Beauv. which had been collected in Tokyo, October 6, 1959.

As experimental germinating bed, 6.5 cm. diameter Petri dish with about 10 ml. of 0.7–0.8% agar solution was used. On the solidified surface, a small amount of water was added, and 100 seeds were disseminated into each Petri dish. Each dish was wrapped in thick black paper immediately, and placed in an incubator, which was usually controlled within $\pm 1^\circ$. In all experiments, the seeds were maintained in darkness throughout, except during the periods of irradiation.

Main light periods were generally supplied as cool white fluorescent light of about 1500 lux. The red or blue radiation was given from cool white fluorescent lamps, through a filter of two layers of red or blue cellophane, respectively, and incandescent light filtered through water and red and blue cellophane was the source of far-red radiation.

Temperature was always controlled at 30° . Two lots of 100 seeds were used for each treatment and the same experiment was repeated several times so as to make sure of the results.

Results

Preliminary experiments concerning the effects of light on germination indicated

* Botanical Institute, Faculty of Science, Tokyo University of Education, Otsuka, Tokyo, Japan.

that seeds of *Eragrostis ferruginea* Beauv. exhibited low germination in continuous exposure to light, although light was obligatory for their germination. But, germination was high even under a long light period if the seeds were given favorable lengths of dark period immediately after soaking (Table 1).

Table 1. Effects on germination of various lengths of darkness immediately after soaking. Light was given for 72 hrs. after dark period, till germination percentages were counted.

Lengths of darkness (hr.)	0	6	12	18	24	36	48	72
Germ. (%)	24	27	49	60	72	70.5	55.5	12.0

This experiment indicated that the length of the dark period was probably the critical factor for germination, as it was in most photoperiodic phenomena.

To observe the optimum length of this dark period, the seeds which had been soaked in water for various durations at 30° in the dark were exposed to light for various lengths (Fig. 1). The effect of irradiation increased rapidly during the first 24 hrs. after the beginning of imbibition. And then the germination percentages decreased gradually with further increase of the dark imbibition time. These experimental results showed that the dark period of 24 hrs. was necessary to obtain the highest germination and its effect might be compared with that of the dark period on the flowering of short-day plants.

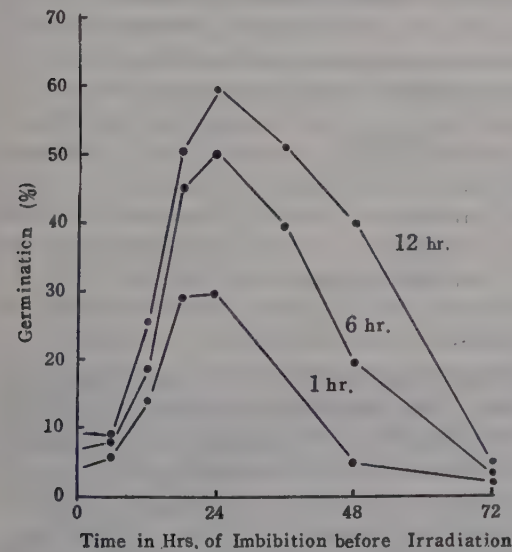


Fig. 1. Light-sensitivity of seed, exposed for 1, 6 and 12 hrs. to 1500 lux.

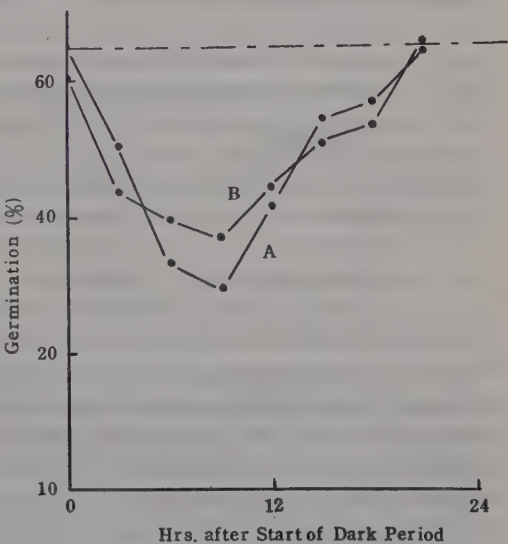


Fig. 2. Effect on germination of a light-interruption of red for 1/6, or blue for 1/6 hr. given at various times in the 24 hr. dark period.

A: Red light B: Blue light

Flowering of short-day plants can be inhibited even under the favorable photo-periods, if the long dark period is interrupted with light. Germination of *Eragrostis* seeds was also inhibited when a brief light interruption was applied during the dark

period (Figs. 2 and 3). Namely, red for 1/6, blue for 1/6 or far-red for 1 hr. was given at various times in the dark period, and the subsequent light period of 12 hrs. was given after the 24 hr. dark period. As shown in Fig. 2, the light-break with red or blue light was most effective when given at about the 9th hr. after the start of the dark period. It was observed, however, that the interruption with far-red light became more effective in the latter half of the dark period (Fig. 3).

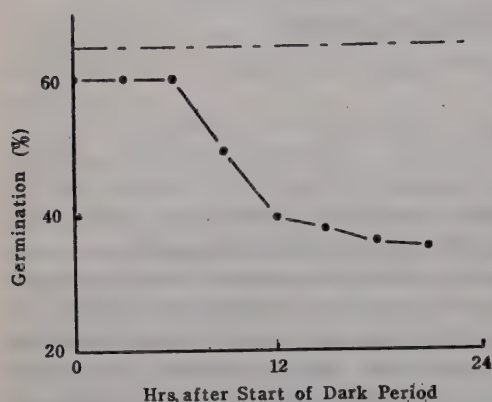


Fig. 3. Effect on germination of a light-interruption of far-red for 1 hr. given at various times in the 24 hr. dark period.

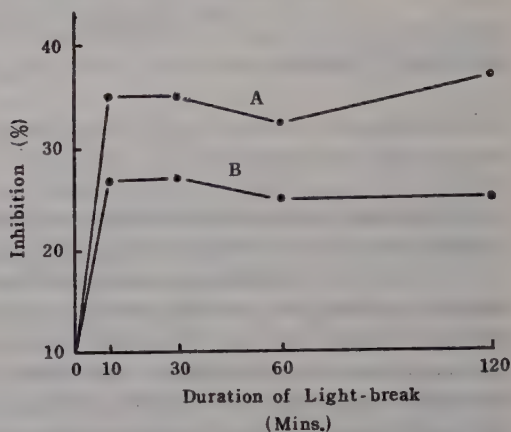


Fig. 4. Effect on germination of various lengths of light interruptions given at 8 hrs. after the beginning of dark period.

A: Red light B: Blue light

Table 2. Effect of alternate exposures to red and far-red or to blue and far-red irradiations given at the 8th hr. after the dark period started.

Treatment	Germ. %	Treatment	Germ. %
R	30.0	B	42.0
R—FR	57.0	B—FR—	50.5
R—FR—R	32.0	B—FR—B	40.5
R—FR—R—FR	56.0	B—FR—B—FR	52.0
—FR—	55.5		

In the next experiment, the dark period was interrupted with various lengths of irradiation when 8 hrs. elapsed from its start. The results are shown in Fig. 4. A light-break of only 10 minutes was effective in inhibiting the germination. However, these inhibitions were reversed by far-red irradiation given immediately after the red or blue light and the germination was controlled by the light last received (Table 2). In this experiment, red, blue and far-red lights were given for 1/6 hr., respectively.

On the other hand, the inhibitive effect of far-red irradiation in the latter half of the 24-hr. dark period was not reversed by red or blue light.

The present results clearly indicated that the germination of *Eragrostis* seeds was affected in a similar way to the flowering of short-day plants by the light-break given during the dark period.

Discussion

Many works have been reported concerning the light action on seed germination.

Various aspects of these problems were summarized in the recent review by Evenari⁸). A few workers, however, have discussed the requirement of dark period in the germination of seeds.

The seeds of *Eragrostis ferruginea* Beauv. germinated in the presence of dark period immediately after soaking and subsequent light period. These results indicated that light was obligatory for the germination of *Eragrostis* seeds. The germination of *Nemophila insignis* seeds is promoted by short days and strongly inhibited under long days⁷). The response of *Eragrostis* seeds is also affected by the duration of both the light and the dark periods, but particularly by the latter, as postulated by Black and Wareing⁵) and Vaartaja⁹).

Eragrostis seeds, once given an inductive photoperiodic treatment, may be induced to germinate. The optimum dark period was 24 hrs. and its effect corresponded to that on flowering of short-day plants.

In the preliminary high-intensity-light period in photoperiodic response, it has been believed on the basis of the work of Liverman and Bonner¹⁰) and others, that photosynthesis provides substrates necessary for the consummation of the subsequent dark steps. It is noteworthy, however, that the dark process commence without preliminary light process in the germination of *Eragrostis* seeds.

The dark process is different from the dark imbibition prior to irradiation in the light-sensitive seed, because the germination is inhibited if the inductive dark period is blocked with a short irradiation of red or blue light. Earlier workers reported that the seed of *Phacelia tanacetifolia* is inhibited by both blue and red lights^{11,12}). And it was found that *Nemophila* seed is inhibited by blue light, but only slightly inhibited under the filters peaking at 542, 547, 596 and 651 m μ . In *Eragrostis* seeds the light-break is most effective when given at about the 9th hr. after the dark period started.

It has been observed that germination of *Lactuca sativa* var. Grand Rapids seeds was either promoted or inhibited in the blue region after the pigment system had been displaced to an extreme of potential inhibition or promotion of germination, respectively¹³). It is noteworthy, however, that the light interruption with blue light is effective at the same part of the dark process as the red interruption is effective, although the effect is less in the former.

The dark process is also blocked with a short irradiation with far-red energy, but its inhibitive effect appears in the latter half of the dark period.

Responses of plant materials to irradiation indicate that germination and many other aspects of development are controlled by a reversible photoreaction^{14,15,16}), involving two forms of a pigment, with action maxima near 6600Å and 7350Å. In *Eragrostis* seed, this photoreversible action is also observed in the first half of the dark period. But in the latter half, the inhibitive effect with far-red is not reversed by red or blue light.

Moreover, Borthwick et al. were able to obtain only slight reversal of the promotive effect of red when the latter was followed by blue¹³). In order to explain the promotive and inhibitory effects of blue light, Borthwick et al.¹³) have postulated that the photoreceptors for the red and far-red responses must have absorptive regions in the blue which overlap. It is highly interesting that the germination of *Eragrostis* seeds is photoreversibly controlled by blue and far-red energies. These have suggested that new insights into the process of germination would be forthcoming from such studies.

Summary

1. Light is obligatory for the germination of *Eragrostis* seeds but the dark period

corresponding to that in flowering of short-day plants is required immediately after soaking. And the high-intensity-light process prior to the dark process is not observed. Germination is maximal under the dark period of 24 hrs. followed by light and decreases under continuous light irradiation.

2. The germination-promoting effects of the long dark period are inhibited by brief interruptions with red, blue or far-red energy, such interruptions being most effective for red and blue at the 9th hr. after the start of the dark period, and for far-red energy in the latter half of the dark period.

3. Repeated alternations of red and far-red radiant energies repeatedly result in red inhibition and far-red repromotion of germination in the first half of the dark period, but not in the latter half.

4. Another important feature is the photocontrol of germination by a succession of alternate blue and far-red irradiations in the first half of the dark period. The germination is regulated by the light last received, being inhibited with blue energy and repromoted with far-red.

References

- 1) Isikawa, S., and Shimogawara, G., J. Japan Forestry Soc. **36**: 318 (1954). 2) Nagao, M., Esashi, Y., Tanaka, T., Kumagai, T., and Fukumoto, S., Plant and Cell Physiol. **1**: 39 (1959). 3) Vaartaja, O., Phytopathology **42**: 501 (1952). 4) Isikawa, S., Bot. Mag. Tokyo, **67**: 51 (1954). 5) Black, M., and Wareing, P. F., Physiol. Plantarum **8**: 300 (1955). 6) Black, M., Ph. D. thesis. University of Manchester, England. (1957). 7) Black, M., and Wareing, P. F., Nature **180**: 395 (1957). 8) Evenari, M., Radiation Biology **111**: 519 (1956). 9) Vaartaja, O., Canada. Jour. Bot. **34**: 377 (1956). 10) Liverman, J., and Bonner, J., Botan. Gaz. **115**: 121 (1953). 11) Meischke, D., Jahr. Wiss. Botan., **83**: 395 (1936). 12) Resühr, B., Planta **30**: 471 (1939). 13) Borthwick, H. A., Hendricks, S. B., Parker, M. W., Toole, E. H., and Toole, V. K., Bot. Gaz. **115**: 205 (1954). 14) Borthwick, H. A., Hendricks, S. B., Parker, M. W., Toole, E. H., and Toole, V. K., Proc. Nat. Acad. Sci. **38**: 662 (1952). 15) Borthwick, H. A., Hendricks, S. B., and Parker, M. W., Proc. Nat. Acad. Sci. **33**: 929 (1952). 16) Isikawa, S., and Fujii, T., Plant and Cell Physiol. (in the press).

摘 要

石川茂雄・藤伊正・横浜康継： カゼクサ種子の発芽における光週期的処理

1) 種子の発芽において、発芽過程を促進する暗期の積極的効果は、いまだ見出されていないが、カゼクサの種子の発芽は短日植物における花芽形成と同様に暗期が存在することによって促進される。しかもこの暗期は播種と同時に始まる。したがって、短日植物におけるような **high-intensity-light process** は観察されなかった。

2) この暗期の発芽促進効果は、**red**, **blue**, および **far-red** の各波長の光の短時間照射によって抑制され、しかも **red** と **blue** の光はともに暗期の9時間目のところでもっとも強い抑制を示す。一方 **far-red** の抑制効果は、暗期の後半において初めて現われてくる。

3) 暗期の前半における **red** と **blue** の抑制効果は **far-red** の光によってとりのぞかれ、この抑制と促進とは何回もくりかえすことができる。しかし暗期の後半における **far-red** の抑制効果は、**red** または **blue** によってとりのぞかれることはなかった。(東京教育大学理学部植物学教室)

A Study on Respiration of Bean Seed Embryo

by Masahiro SUGIURA*

Received July 11, 1960

A bean seed embryo is divided into two parts of metabolic as well as morphological differentiation, i.e., anabolic germ-axis and catabolic cotyledons. It has been suggested that this anabolic or catabolic nature of a given tissue of the embryo is determined by energy-rich phosphate level which, in all likelihood, will be most closely connected with respiratory activity of the tissue concerned^{1,2}). The previous works in our laboratory have shown that cytochromes *a*, *b* and *c*³) and a whole set of component dehydrogenases of Krebs-cycle system are present in germ-axis⁴), and that, at least in the early stage of germination, cotyledon contains cytochromes *a*₁ and *b*, but no *c*, together with nitrate reductase which can function as a terminal oxidase^{3,5}). Weak activity of cytochrome oxidase detected in cotyledon at the outset of germination grows up gradually with the age of the tissue⁶). It is of interest that this increase in oxidase activity is accompanied by the appearance of cytochrome *c*. Although cotyledon has relatively strong activity of alcohol and formic dehydrogenases, it contains no detectable activity of citric dehydrogenase⁴) (see also a detailed discussion by Yamamoto⁵) on the anaerobic nature of cotyledonous formic dehydrogenase). These studies suggest that the germ-axis tissues are equipped with typical aerobic type of respiratory system and that in the early period of germination the cotyledon tissues have redox system more or less characteristic to the facultative anaerobes²).

In the present study, the respiratory rates (Q_{O_2}) and the respiratory quotients (RQ) of germ-axes and of various portions of cotyledons isolated from soaked bean seeds were estimated.

The endogenous carbohydrate respiration ($RQ=1$) of germ-axes and that of inner portion of cotyledons were also compared with respect to their sensitivity to several metabolic poisons. The findings reported below provide us with a further insight into the difference in respiratory machinery between the growing and the degenerating tissues of the germinating seeds.

Materials and Methods

Bean seeds. Seeds of *Vigna sesquipedalis* stored in a dark desiccator for about a year after harvest were used. Germ-axes or cotyledons were separated from the seeds presoaked in tap water for 6 hours at 30°. Cotyledon as a whole and inner part (ca. 2×4×6 mm. block) were cut into slices approximately 0.8 mm. (11–15 cells) thick with a cutting device made of razor blades (hereafter the former preparation is referred to as **whole slices** and the latter **inner slices**). The outermost part of cotyledon was peeled from which **outer slices** (ca. 0.3 (1–6 cells) ×2×6 mm.) were prepared. The intact germ-axes, intact cotyledons and three sorts of cotyledon slices thus obtained were rinsed repeatedly with distilled water, and blotted well with filter paper before use.

Gas exchange. Gas exchange was investigated at 30° in the dark using conventional Warburg respirometers. Ten germ-axes, five to ten intact cotyledons or 200–500 mg. of the cotyledon slices were placed in the main room. One and a half or

* Biological Institute, Faculty of Science, Nagoya University, Chikusa, Nagoya, Japan.

2.0 ml. of a phosphate buffer (M/30, pH 6.0, unless otherwise stated) was pipetted into the main room of every respirometer which was then immediately immersed in a water bath at 30°. After a 15 min. period of temperature-equilibration manometric readings were started and continued over a period of 90–210 min. The direct method was applied for the CO₂ estimation⁷⁾. In the anaerobic experiments, air was replaced with argon by the procedure described by Umbreit *et al.*¹⁾ After the experiment of gas exchange was finished, the samples used were removed from the flasks, rinsed with distilled water and dried at 95–105° to constant weight to obtain dry weight. The Q_{O₂} and the Q_{CO₂} were calculated on a dry weight basis.

Application of poisons. One half ml. of poison solution dissolved in a phosphate buffer (M/30, pH 6.0) was tipped from the side arm into the main room 50 min. after the beginning of the reading and the effect of poison was examined for further 40 to 160 min. In the control run, 0.5 ml. of the phosphate buffer was added in place of poison solution. In cyanide experiments, a KOH-KCN mixture as a CO₂ absorbent was used and 0.5 ml. of aqueous KCN solution was added just before starting the measurement. In the control run, aqueous KCN solution was replaced with the same volume of distilled water. Oxygen uptake in the first 120 min. was determined. The pH of the medium (in this case M/15 phosphate buffer, pH 6.0, was used) was raised slightly by the addition of KCN solution. Sodium diethyldithiocarbamate (dieca) was examined at pH 7.0 considering its instability in acid solution⁸⁾. For carbon monoxide experiment, cotyledons were cut into thinner slices (ca. 0.4 mm. thick) and germ-axes (ca. 8 mm. long) were also cut transversely into four pieces of ca. 2 mm. length so as to enable oxygen to diffuse readily into the tissues placed under 5 vol. per cent oxygen. Gas phase of the control run contained 95 vol. per cent of argon and 5 vol. per cent of oxygen. Photoreversibility was examined by illuminating the bottom of the flasks with two 100 watt incandescent lamps from 18 cm. distance.

Further details will be given in the legends of respective tables and figures.

Table 1. Gas exchange of germ-axes and various parts of cotyledons. Warburg respirometers used. 10 germ-axes, 5–10 cotyledons, 300 mg. **whole slices**, 250 mg. **outer slices** or 300 mg. **inner slices** per flask containing 2.0 ml. of M/30 phosphate buffer, pH 6.0. Air; 30°; in the dark.

	A				B		C	
	Q _{O₂}	Q _{CO₂}	RQ	resp. rise	carbohydrate resp.	fat resp.	$\frac{Q_{\text{argon CO}_2}}{Q_{\text{CO}_2}}$ (1st 1 hr.—2nd 1 hr.)	I/N
Germ-axes	7.8	7.8	1.0	0	—	—	7.0–6.3	0.90–0.60
Intact cotyledons	0.52	0.42	0.81	0	0.19	0.33		
Whole slices	0.72	0.62	0.89	44	0.45	0.27		
Outer slices	1.30	1.07	0.82	53	0.53	0.77		
Inner slices	0.44	0.42	0.96	54	0.37	0.07	0.31–0.21	0.70–0.42

A: The initial rates of gas exchange (μl./mg. dry weight/the first one hour) and the respiratory rise (% of the initial rates) at 3 hours (cf. Fig. 1).

B: Compositions of the Q_{O₂} obtained theoretically.

C: Anaerobic CO₂ production (μl./mg. dry weight/hour) and I/N ratios ($\frac{Q_{\text{argon CO}_2}}{Q_{\text{CO}_2}}$) measured for the first and the second 1 hour.

Results

Gas exchange. The rates of O₂ uptake and CO₂ output of the tissue preparations

are shown in Table 1 (column A). The Q_{O_2} of germ-axes is seen to be much higher than that of every cotyledon preparation examined. In germ-axes, the rate of O_2 uptake remained constant for at least 3 hours, but the rate of CO_2 output, which was initially equal to the rate of O_2 uptake, rose suddenly at about 60 min., resulting in an increase of the RQ from ca. 1.0 to ca. 1.4 (Fig. 1-A). In the case of cotyledons, the rate of either O_2 uptake or CO_2 output of intact tissues was found to be kept constant for at least 3 hours, while that of slices, either **whole**, **outer** or **inner slices**, began to rise at about 100 min. until it was increased by about 50 per cent in 3 hours.

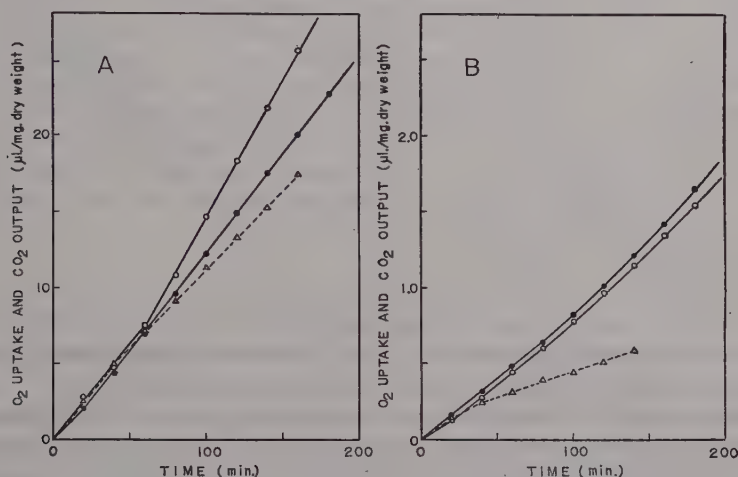


Fig. 1. Gas exchange of germ-axes (A) and **inner slices** of cotyledons (B). Solid circles, O_2 uptake; open circles, CO_2 output; triangles, anaerobic CO_2 output. See the legend of Table 1 for further explanation.

In Fig. 1-B only the results obtained for **inner slices** are illustrated, since other slices yielded similar results. Noteworthy differences in respiration among three preparations of cotyledons are also indicated in Table 1 (column A). Oota *et al.*⁹) have suggested that the isolated bean cotyledons can utilize simultaneously two respiratory substrates, i.e., carbohydrate and fat. Standing on this view, the share taken by each of these two substrates in the cotyledon respiration was calculated from the data shown in Table 1 (column A), and the results are given in the same Table (column B). Thus it is clear that a higher respiratory rate obtained for **whole slices** than that obtained for intact cotyledons is attributed merely to an accelerated carbohydrate respiration due probably to slicing. It is striking that fat respiration was affected little by tissue slicing. Moreover, the Q_{O_2} of **outer slices** was about three times as high as that of **inner slices** and Table 1 (column B) shows that the former uses predominantly fat and the latter, like germ-axes, nearly exclusively carbohydrate as respiratory substrates. As expected, the Q_{O_2} and the RQ values of **whole slices** lie between those of **outer** and **inner** ones, respectively.

Effects of sodium fluoride and monoiodoacetate. It was noticed above that of cotyledon preparations **inner slices** alone are nearly indistinguishable from germ-axes as to the quality of respiration as judged from their RQ values ($RQ \approx 1$). Both are likely to respire carbohydrate. It will be of interest, therefore, to examine whether the respiration of germ-axes and that of **inner slices** of cotyledons are affected similarly or not by various respiratory poisons.

The gas exchange of germ-axes was increasingly inhibited by 2.5×10^{-2} M sodium

fluoride (NaF) (Fig. 2-A). The inhibition of O_2 uptake came earlier than that of CO_2 output did. The CO_2 output/time curve, however, became parallel with the O_2 uptake/time curve (the RQ reached unity) about an hour after the addition of NaF, in other words, the extra CO_2 production as observed in the control run or in the initial phase of NaF action perfectly disappeared.

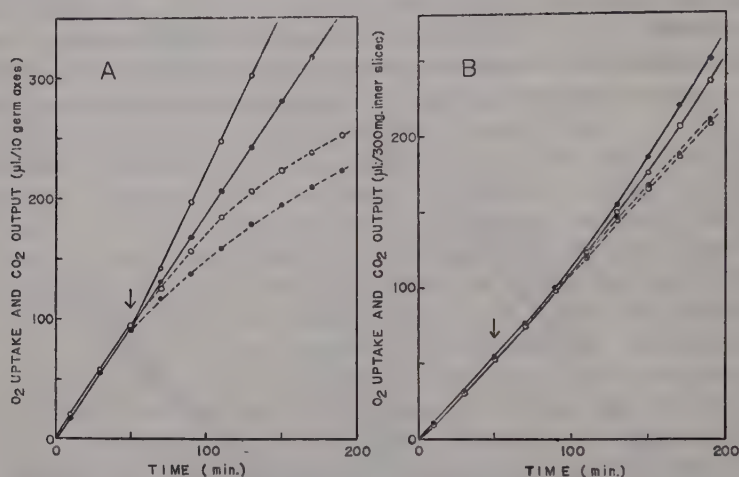


Fig. 2. Effect of NaF ($2.5 \times 10^{-2} M$) on gas exchange of germ-axes (A) and **inner slices** of cotyledons (B). Arrows indicate the addition of NaF. Solid circles, O_2 uptake; open circles, CO_2 output; solid lines, no NaF; broken lines, $2.5 \times 10^{-2} M$ NaF. See the legend of Table 2 for further explanation.

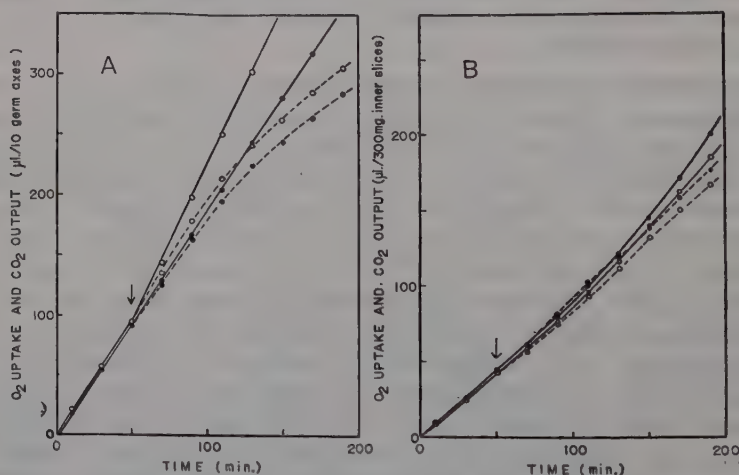


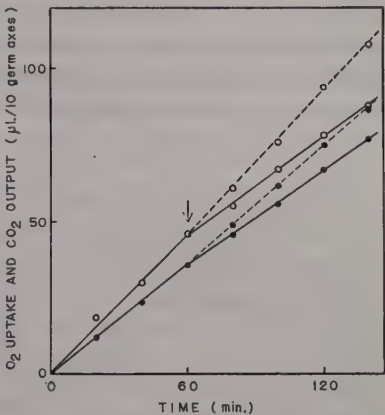
Fig. 3. Effect of MIA ($10^{-3} M$) on gas exchange of germ-axes (A) and **inner slices** of cotyledons (B). Arrows indicate the addition of MIA. Solid circles, O_2 uptake; open circles, CO_2 output; solid lines, no MIA; broken lines, $10^{-3} M$ MIA. See the legend of Table 2 for further explanation.

An enhancement of gas exchange of **inner slices** began to proceed at about 100 min., as mentioned earlier. And now this rise was completely diminished by $2.5 \times 10^{-2} M$ NaF, remaining the basal respiratory activity unaffected (Fig. 2-B).

Moniodoacetate (MIA, $10^{-3} M$) yielded very similar effect to $2.5 \times 10^{-2} M$ NaF, as shown in Fig. 3.

As shown in Fig. 4, the addition of 5×10^{-2} M pyruvate was found to cause partial recovery of NaF inhibition in germ-axes, i.e., 9 and 19 per cent recoveries for O_2 uptake and for CO_2 output, respectively, and the RQ was raised from 1.13 to 1.31, while

Fig. 4. Effect of pyruvate (5×10^{-2} M) on gas exchange of NaF-treated germ-axes. Warburg respirometers used. 10 germ-axes per flask containing 1.5 ml. of 2.5×10^{-2} M NaF—M/30 phosphate buffer, pH 6.0. Air; 30° ; in the dark. Measurement was started 70 min. after the addition of NaF, when the inhibitory effect of the reagent was wholly manifested (see Fig. 2-A). Arrow indicates the addition of sodium pyruvate dissolved in a phosphate buffer (M/30, pH 6.0). Solid circles, O_2 uptake; open circles, CO_2 output; solid lines, no pyruvate; broken lines, 5×10^{-2} M pyruvate.



that of the control run (NaF inhibited respiration) dropped from 1.13 to 1.00.

Effects of heavy metal reagents. As summarized in Table 2, O_2 uptake of either germ-axes or **inner slices** was almost completely inhibited by higher concentrations of cyanide than 10^{-3} M. The poisonous effect of sodium azide (NaN_3) was also remarkable

Table 2. Effect of heavy metal reagents on O_2 uptake of germ-axes and **inner slices** of cotyledons. Warburg respirometers used. 10 germ-axes or 300–500 mg. **inner slices** per flask containing 1.5 ml. (2.0 ml. for CO) of M/30 (M/15 for KCN) phosphate buffer, pH 6.0 (pH 7.0 for dieca). Air (excepting CO experiments); 30° ; in the dark.

Inhibitor	conc. (M)	Inhibition (%)	
		germ-axes	cotyledons
KCN	1×10^{-4}	91	83
	1×10^{-3}	100	88
	1×10^{-2}	100	91
NaN_3	1×10^{-4}	48	39
	1×10^{-3}	66	70
	5×10^{-3}	—	69
CO	95% dark	31	62
	light	0	18
dieca	5×10^{-3}	8	27
salicylaldoxime	5×10^{-3}	40	13
α, α' -dipyridyl	2×10^{-3}	26	40
<i>o</i> -phenanthroline	2×10^{-3}	44	17

but the inhibition did not exceed 70 per cent by the use of up to 5×10^{-3} M of the reagent (see also Fig. 6). Oxygen uptake of **inner slices** was also inhibited strongly by 95 vol. per cent CO in the dark and the inhibition was largely removed by light. Carbon monoxide gave unexpectedly low inhibition to the respiration of germ-axes, and the inhibition was removed completely by light.

Both dieca and salicylaldoxime (5×10^{-3} M) inhibited O_2 uptake rather slightly;

germ-axes were more sensitive to the latter than to the former in contrast to a higher sensitivity of **inner slices** of cotyledons to the former.

By the way, the addition of α , α' -dipyridyl or *o*-phenanthroline (2×10^{-3} M) as the reagents capable of chelating with iron atom did not give any immediate effect, but induced a partial suppression of respiration with the lapse of time. Cotyledon slices were about two times as sensitive to α , α' -dipyridyl as to *o*-phenanthroline, while reversely germ-axes were much more sensitive to the latter. The exact mechanism of the action of these reagents is obscure.

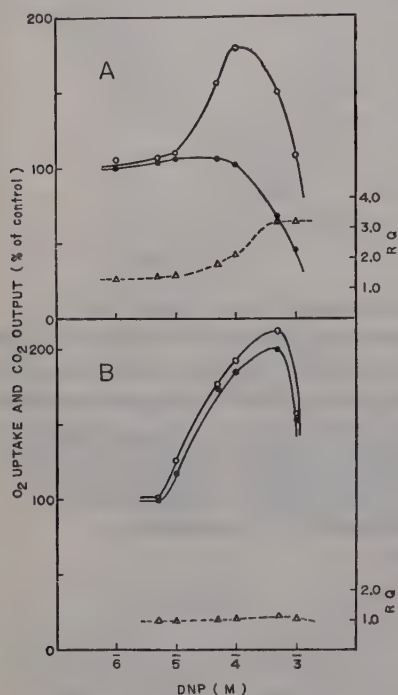


Fig. 5. Effect of varied concentrations of DNP on O₂ uptake and CO₂ output of germ-axes (A) and **inner slices** of cotyledons (B). Solid circles, O₂ uptake; open circles, CO₂ output; triangles, RQ. See the legend of Table 2 for further explanation.

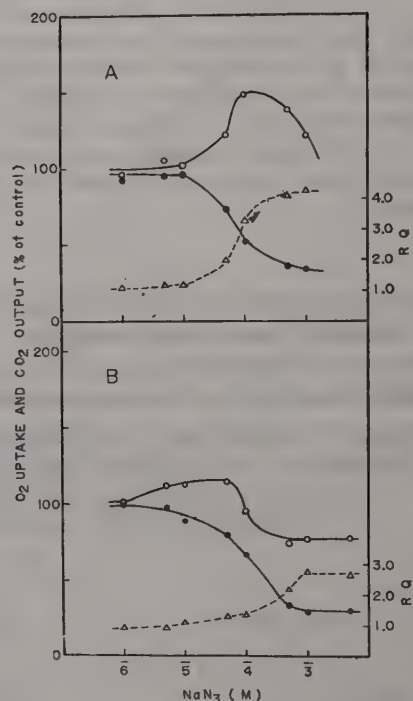


Fig. 6. Effect of varied concentrations of NaN₃ on O uptake and CO₂ output of germ-axes (A) and **inner slices** of cotyledons (B). See the legends of Table 2 and Fig. 5 for further explanation.

Effects of uncoupling reagents. Striking difference between germ-axes and cotyledons was found as for their response to 2, 4-dinitrophenol (DNP). Oxygen uptake of germ-axes was affected little by low concentrations of DNP, but a marked inhibition was induced by concentrations greater than 10^{-4} M (Fig. 5-A). On the other hand, the CO₂ output/DNP concentration curve showed a definite peak at 10^{-4} M of DNP, where the stimulation amounted to about 80 per cent of the control. After the peak was passed the curve declined abruptly. Thus the RQ was markedly increased in the presence of DNP at concentrations greater than 10^{-5} M. In cotyledons (Fig. 5-B), however, DNP at such a low concentration as 10^{-5} M induced a pronounced stimulation of O₂ uptake; at 5×10^{-4} M the stimulation was the highest amounting to about 100 per cent of the control. Thereafter, the curve also dropped sharply. Carbon dioxide

output showed a simultaneous rise and fall with O_2 uptake, and the RQ was maintained roughly at unity over the whole range of concentration examined.

Assuming that DNP added does not change the quality of original respiration¹⁰), i.e., $RQ=1$ is maintained unchanged in cotyledon slices and germ-axes in the presence of DNP, the difference between the CO_2 and the O_2 curves in Fig. 5 may be directly regarded as the amount of extra CO_2 output in terms of percentage of the control CO_2 output. It is likely that DNP at concentrations greater than 10^{-5} M may insert strong aerobic fermentation in the gas metabolism of germ-axes, whereas the gas exchange of cotyledon slices consists practically solely of respiration even under the presence of DNP.

Similar experiments with NaN_3 gave the results shown in Fig. 6. In germ-axes, the effect observed was very similar to that of DNP. Oxygen uptake was inhibited by NaN_3 at concentrations greater than 10^{-5} M, which evoked a considerable stimulation of CO_2 output. The effect of NaN_3 on cotyledon slices was apparently different from that of DNP. The greater the NaN_3 concentration, the more striking was the inhibition of O_2 uptake. In contrast with this, the CO_2 output was accelerated slightly by lower and decreased by higher concentrations than 10^{-4} M. It must be pointed out, however, that the CO_2 output curve runs nearly parallel with the O_2 uptake curve as was the case for the DNP experiment. Assumingly the responses of these seed tissues to NaN_3 and DNP are essentially the same, and to the known inhibitory action of the former on oxygen respiration (cf. Table 2) will be ascribed the above described slight difference between the gas exchange/ NaN_3 and the gas exchange/DNP curves.

Discussion

The Q_{O_2} of germ-axes was very much greater than that of the cotyledon tissues. The Q_{O_2} values, however, were given on a dry weight basis, so they could not permit us to compare directly the respiratory activities of the tissues in question, since the dry matters of the cotyledon tissues should contain considerable amount of reserve materials.

The results of the NaF and MIA experiments suggest the participation of Embden-Meyerhof system in the respiration mechanism of the germ-axis tissues. The presence of cytochromes *a*, *b* and *c* and of Krebs cycle dehydrogenases in germ-axes has been shown previously^{3,4}), and it was now demonstrated that their respiration was strongly sensitive to a variety of iron reagents. From these it may be inferred that the respiration of germ-axes is a typical carbohydrate respiration involving Embden-Meyerhof pathway, Krebs cycle and cytochrome *c*/cytochrome oxidase system. The RQ value of germ-axes rose from unity to 1.4 after about an hour of shaking. This rise may be due to the occurrence of fermentation since the increased RQ value was lowered again to unity by the addition of NaF or MIA and the effect of the former could be removed by the addition of pyruvate.

There are significant differences in quality as well as in quantity of respiration between outer and inner parts of cotyledon. Isolated outer part respired much more strongly ($Q_{O_2}=1.30$) than isolated inner part did ($Q_{O_2}=0.44$) and the former appeared to utilize fat ($RQ=0.82$) and the latter carbohydrate ($RQ=0.96$) as their preferential respiratory substrates. In view of the suggestion of Oota *et al.* that the formation of sucrose from starch in germinating cotyledon is backed up by fat respiration⁹), sucrose may assumingly be formed predominantly in outer part of cotyledon. In fact, Kawamatu has shown cytochemically the preferential disappearance of starch granules in this area of germinating cotyledons of *Vigna sesquipedalis*¹¹).

There seems to be no difference as to the main terminal respiratory system between germ-axes ($RQ \approx 1$) and inner part of cotyledon ($RQ \approx 1$), since the respiration of **inner slices** was shown to be as sensitive to the iron reagents as that of germ-axes was. Contradictory to this, at least in the early germination stage, intact cotyledons have been reported to contain cytochromes a_1 , b and b_4 but no c and little cytochrome oxidase activity^{8,12}). At the same time, however, it is known that this b_4 component can be converted readily into cytochrome c by mere aeration of the tissues¹²). Interesting to say, the conversion of b_4 to c accompanies the appearance of the oxidase activity²). It sounds, therefore, reasonable to conclude that the slicing of the tissues would bring a sufficient supply of atmospheric oxygen to the tissues resulting a change in their respiratory pattern, i.e., the establishment of cytochrome c /cytochrome oxidase system. This can also explain at least partly the fact that **whole slices** respire at a greater rate than intact cotyledons do (see Table 1, column A).

In contrast with the case of germ-axes, NaF or MIA did not affect the basal respiration of **inner slices**, but removed completely the respiratory rise which was to come after 100 min. Accordingly the developed part of respiration may involve Embden-Meyerhof system, while the basal one may not. According to Oota *et al.*, Krebs cycle system is unlikely operative in cotyledons because of the absence of any detectable citric dehydrogenase activity in their tissue homogenates⁴). Further analyses are needed to elucidate the pathway of carbohydrate breakdown in the cotyledon tissues.

Copper reagents examined induced respiratory inhibition in both tissues, suggesting that some copper enzyme(s) may also play the rôle of terminal oxidase(s). To date little information is available as for copper enzymes of the present materials²).

It is widely accepted that DNP uncouples phosphorylation from oxidation, and elevates the level of phosphate acceptor (ADP) which should be in an intimate connexion with the respiratory activity of the tissues. Observed stimulation of respiration of the cotyledon tissues (**inner slices**) induced by DNP indicates that the phosphorylative capacity may limit the rate of the respiration in question. In contrast, little DNP effect observed for O_2 uptake of germ-axes suggests that the situation is quite different in the growing tissues, that is, the rate of respiration of the tissues is hardly limited by the ADP level. Nevertheless, it is possible that the ADP level elevated by the action of DNP may speed up the carbohydrate breakdown if the rate of breakdown is limited by the ADP level. Then, if the supply of the product of carbohydrate breakdown thus accelerated exceeds the capacity of the tissues of consuming it by aerobic means, aerobic fermentation or extra CO_2 production may occur, as was the case for the germ-axes used (Fig. 5-A). It is supposed that in cotyledons the increased supply, if any, due in some way to the effect of the uncoupler added will be readily utilized by the promoted respiration.

Relevant to the fact that in germ-axes DNP induced strong aerobic fermentation, I/N ratio obtained for the tissues was found to be higher than that obtained for the cotyledon slices, i.e., the former tissues manifest the Pasteur effect more notably than the latter do (Table 1, column C).

Summary

1. Gas exchange (in a phosphate buffer, pH 6.0, M/30) of intact germ-axes and slices of various parts of cotyledons isolated from soaked seeds of *Vigna sesquipedalis* were examined. The Q_{C_2} on a dry weight basis and the RQ were found to be 7.8 and 1.0 for germ-axes and 0.52 and 0.81 for intact cotyledons, respectively. The Q_{O_2} of outer part of cotyledons (1.30) was strikingly higher than that of inner part (0.44)

and the RQ of the former (0.82) was lower than that of the latter (0.96).

2. Sodium fluoride and monoiodoacetate inhibited gas exchange of germ-axes strongly, but affected little that of inner part of cotyledons.

3. Cyanide and azide inhibited O_2 uptake of both germ-axes and inner part of cotyledons much strongly. Oxygen uptake of inner part of cotyledons was inhibited by carbon monoxide more considerably than that of germ-axes. The inhibition was completely (in the latter tissues) or almost completely (in the former) removed by light. Partial suppression of O_2 uptake of these two tissues was also evoked by the addition of dieca, salicylaldoxime, α , α' -dipyridyl or *o*-phenanthroline.

4. 2, 4-dinitrophenol stimulated O_2 uptake little and CO_2 output considerably of germ-axes, whereas it stimulated strongly both O_2 uptake and CO_2 output of inner part of cotyledons. Essentially similar results were obtained with azide.

5. Gas exchange machineries of the germ-axis and the cotyledon tissues were briefly discussed.

The author wishes to express his sincere gratitude to Assoc. Prof. Y. Oota, under whose guidance this study has been carried out. His thanks are also due to Dr. M. Izawa for his helpful advice and discussions.

References

- 1) Oota, Y., *Kagaku* **23**: 60 (1953).
- 2) — —, Biochemistry of seed germination, in *Biochemistry of life phenomena* (Ed. by Ashida, J., *et al.*), Asakura Publ. Co., Tokyo (1955).
- 3) Kumada, H., *J. Biochem.* **40**: 439 (1953).
- 4) Oota, Y., Yamamoto, Y., and Fujii, R., *ibid.* **40**: 187 (1953).
- 5) Yamamoto, Y., *ibid.* **41**: 551 (1954).
- 6) Oota, Y., Yamamoto, Y., Fujii, R., and Yaguchi, H., reported at the Annual Meeting of the Botanical Society of Japan in 1950.
- 7) Umbreit, W. W., Burris, R. H., and Stauffer, J. F., *Manometric Techniques and Tissue Metabolism*, Burgess Publ. Co., Minneapolis (1949).
- 8) James, W. O., and Garton, N., *J. Exp. Bot.* **3**: 310 (1952).
- 9) Oota, Y., Fujii, R., and Sunobe, Y., *Physiol. Plantarum* **9**: 38 (1956).
- 10) Gaur, B. K., and Beevers, H., *Plant Physiol.* **34**: 427 (1959).
- 11) Kawamatu, S., *Bot. Mag. Tokyo* **70**: 152 (1957).
- 12) Mori, T., Kumada, H., and Hirai K., reported at the Annual Meeting of the Botanical Society of Japan in 1954.

摘 要

杉浦昌弘： マメの種子胚の呼吸の研究

1. あらかじめ吸水させたミトリササゲ種子から分離した軸組織と、子葉のいろいろの部分の切片とのガス交換 ($M/30$, pH 6.0 の磷酸塩緩衝液中) を測定した。乾燥量あたりの Q_{O_2} と RQ は軸組織で 7.8 と 1.0, intact 子葉で 0.52 と 0.81 である。子葉の外部組織の Q_{O_2} (1.30) は内部組織のそれ (0.44) より高いが、 RQ は外部 (0.82) の方が内部 (0.96) より低い。

2. Sodium fluoride, monoiodoacetate は軸組織のガス交換を強く阻害するが、子葉内部組織のそれはほとんど阻害しない。

3. Cyanide, azide は軸組織と子葉内部組織とのどちらの酸素吸収もいちじるしく阻害する。一酸化炭素は軸組織の酸素吸収よりも子葉内部組織のそれを強く阻害する。CO 阻害は前者では完全に、後者ではその大部分が光によって除去される。Dieca, salicylaldoxime, α , α' -dipyridyl, *o*-phenanthroline も両組織の酸素吸収を多少阻害する。

4. 2, 4-Dinitrophenol は軸組織の酸素吸収をほとんど促進しないが、炭酸ガス放出をいちじるしく促進する。子葉内部組織では酸素吸収、炭酸ガス放出ともにこの uncoupler によって強く促進される。本質的に同じ結果が azide によっても得られた。

5. これらの結果ならびにさきにわれわれの研究室で得られた結果にもとづいて、軸組織と子葉組織とのガス交換機作について簡単に考察した。(名古屋大学理学部生物学教室)

Changes in Ascorbic Acid Content in Plumules of *Nelumbo nucifera* During Maturation of the Fruit

by Kiyonobu TOYODA*

Received May 19, 1960

In a previous paper¹⁾, the writer has reported the presence of ascorbic acid in the plumule of *Nelumbo nucifera* Gaertn. Based on the results of observations on the changes occurring during the development of the *Nelumbo* fruit, from the earliest period of formation to the full maturity, the writer has distinguished seven developmental stages as described in another paper²⁾. The present paper describes the changes in the content of ascorbic acid (reduced and total) in the plumule of *Nelumbo* fruit which was collected in the lotus field at Kamakura. These experiments were carried out in August, 1959. The usual titration method with 2, 6-dichlorophenol indophenol was used for the determination of ascorbic acid. To examine the initial phase of ascorbic acid formation in detail, the second stage of development was further divided into two steps; namely the early second stage (IIa) and the late one (IIb). In the former stage, the plumule is covered with mucus and is yellow in appearance; in the latter stage, the plumule becomes larger in size and yellowish-green in color. The experimental results are shown in Figs. 1 and 2.

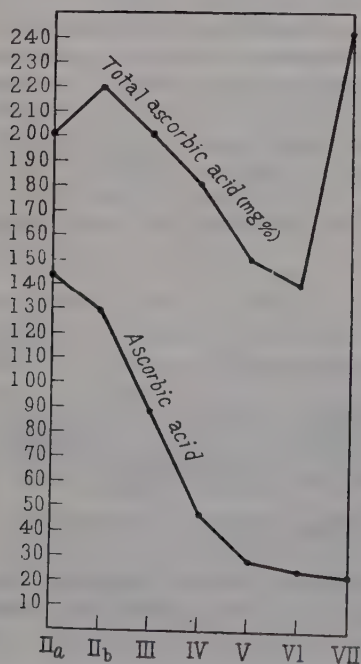


Fig. 1. Content of ascorbic acid (mg. %) in the plumule of *Nelumbo nucifera* in various maturing stages.

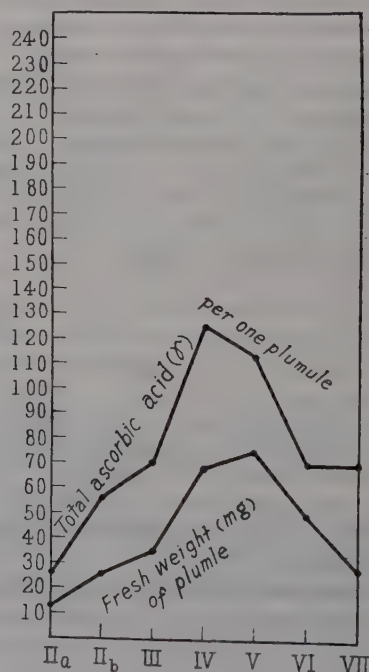


Fig. 2. Average fresh weight (mg.) and total amount of ascorbic acid (γ) per one plumule of *N. nucifera* at various maturing stages.

* Fujisawa Higher School attached to Nihon University, Fujisawa, Kanagawa Pref., Japan.

As will be seen from Fig. 1, the level of ascorbic acid in the plumule (mg. %) is highest in the early stage (IIa) of growth, to decrease gradually with the further development of the fruit. The level of total ascorbic acid (reduced plus dehydro-ascorbic acid) in the plumule reaches a maximum in the late second stage (IIb), later to decrease gradually. At full maturity of the fruit (i.e., Stage VII) there is a marked rise in level of total ascorbic acid, while that of ascorbic acid remains at the lowest stationary state.

The lower line in the Fig. 2 represents the average fresh weight of the plumule, which shows a maximum at the fifth stage of growth. As will be seen from Fig. 2, the total amount of ascorbic acid per one plumule increases with the progress of maturation of the fruit to attain a maximum at the fourth stage, then to show a rapid decrease.

It will be mentioned that these values in the level of ascorbic acid, expressed in mg. per cent on fresh weight basis, are near to those¹⁾ found in the leaves of the same plant. The general trend of the changes described above is in accord with the finding of Hoover³⁾, who has followed the changes in ascorbic acid (reduced form) in Southern pea.

References

- 1) Toyoda, K., Bot. Mag. Tokyo **73**: 98 (1960). 2) —, Jour. Jap. Bot. **33**: 85 (1958). 3) Hoover, M. W., Food Res. **20**: 409 (1955).

摘 要

豊田清修： 成熟中のハスの果実の幼芽におけるアスコルビン酸含有量の変化

成熟中のハスの果実の幼芽におけるアスコルビン酸の含有量をインドフェノール法で測定した。還元型では幼芽形成の初期(第2段階前期)において最高で、のち漸減する。総アスコルビン酸(還元型と酸化型)では第2段階後期において高く、のち漸減、完熟(第7段階)の果実では急増する。幼芽1個あたりの総アスコルビン酸は第4段階で最高に達する。初期における還元型と総アスコルビン酸の含有量はハスの葉におけるそれらに近い。(日本大学藤沢高等学校)

中沼の湖底泥における水生菌類の季節的变化

鈴木 静 夫*

Shizuo SUZUKI*: The Seasonal Changes of Aquatic Fungi
in Lake Bottom of Lake Nakanuma

1960 年 5 月 2 日受付

湖沼において水生菌類が著しい季節的消長を示すことは先に報じたが^{1,2)}, 湖底泥中での消長についてはいまだ知見が得られていない。著者は 1957 年 10 月から翌年 9 月までの 1 年間、茨城県竜ヶ崎町の近くにある中沼³⁾において、湖底泥中の水生菌類の季節的消長を観察したので、その結果を報告する。

研究 方 法

湖泥はエクマン・バージ式採泥器を使用し、湖の最深部の数箇所 20 個の試料を採集した。試料はポリエチレンの袋に入れて実験室に持ち帰り、室温で数日間放置した後に、前報と同じ方法で水生菌類の分離を行なった⁴⁾。

湖底泥中における出現頻度の年変化

湖底泥中の水生菌類の出現頻度は、季節による変化が著しく、1つの最大出現期と1つの最小出現期を持つ (Fig. 1)。すなわち、底層水に酸素が十分に溶存している1月から4月には多量の水生菌類が見

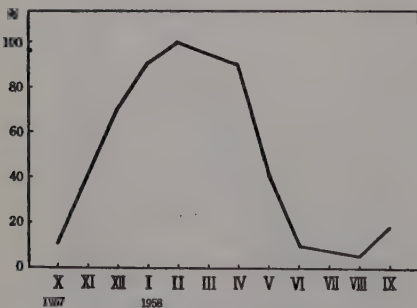


Fig. 1. Seasonal changes of frequency of the aquatic fungi in bottom mud of Lake Nakanuma.

* Department of Microbial Chemistry, Faculty of Pharmacy, Tokyo College of Science, Ushigome, Tokyo, Japan. 東京理科大学薬学部微生物化学教室。

られるが、無酸素層の形成に伴って5月に出現率は減少し始める。水温躍層の発達につれて無酸素層も厚くなり、6月には水生菌類が急激に減少し、この状態は10月までつづき、出現頻度はわずか5~20%にすぎない。12月にはなお底層が無酸素状態にもかかわらず⁵⁾, 出現率は急激に増加し、78%を示す。このように湖底泥中での水生菌類の繁殖は冬季に最大となるのに対し、湖水では春季と秋季に最大となり^{1,2)}, 両者の消長が全く異なることは注目値する。これは後者の消長を支配する要因として水温が、前者の場合には底層水の酸素含有量が主として関係しているためと考えられる。底層水の酸素含有量の年変化は Table 1 に示すとおりで、酸素の消失する時期と水生菌類の減少する時期とがほぼ一致することは、これを裏づけるものといえる。

Table 1. Seasonal changes of water temperature, pH and O₂ in the water of bottom layer of Lake Nakanuma

	1957 X	XII	1958 I	II	
Water temperature (°C) } pH O ₂ (ml/l)	8.9	9.0	7.6	5.6	
	7.0	7.0	7.2	7.3	
	0	0	6.23	8.37	
	1958 IV	V	VI	VIII	IX
	7.0	8.0	8.5	8.2	8.7
	6.9	6.9	7.0	6.9	—
	1.32	0	0	0	0

菌類の消長で特に注目すべきことは、循環期から停滞期に移る場合の変化である。すなわち、湖水の停滞が始まる4月には90%の出現率を示すが、5月になると底層水の酸素の消失とともに43%に、さらに6月には10%にまで減少する。無酸素となった月に多量の菌類が泥より分離されたことは、酸

素が十分に存在した時期に形成された無酸素状態に長時間耐えうる抵抗芽や、卵孢子などが存在していたためであろう。また、12月に底層水が無酸素であるにもかかわらず多量の菌類が見られた原因として、水温の低下によって表層水中の遊走子が膜をかぶって休止し、湖底に堆積したものと思われる。

種類の年変化

湖底泥から分離された水生菌類の種類別の出現頻度を Fig. 2 に示した。得られたものは *Pythium* sp. と *Aphanomyces* sp. を主とする6種で、一

般的に種類数、出現頻度ともに冬季に多く、夏季に減少し、特に8月には少数の *Pythium* sp. がみられるにすぎない。

次に各種類の消長を見ると、*Pythium* sp. は1年を通じてみられる唯一の種で、12月から4月までは40~85%の出現率を示すが、湖底が無酸素となる夏季停滞期には5~15%に減少する。*Aphanomyces* sp. と *Achlya flagellata* の消長は類似しており、底層に酸素が十分に溶存する循環期にだけみられ、夏には全く出現しない。*Achlya* 属は通常湖底泥に10~20%の頻度で分布しているが⁶⁾、

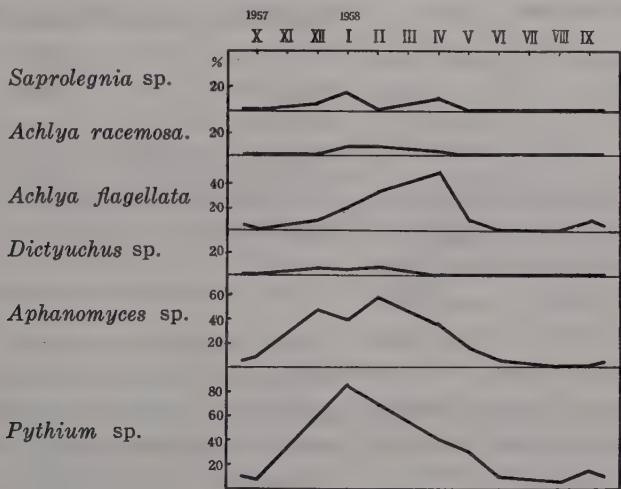


Fig. 2. Seasonal changes of frequency of various species of aquatic fungi in bottom mud of Lake Nakanuma.

Table 2. Effect of oxygen tension on mycelial growth and zoospore formation.

O ₂ ml/l		0.56	7.06
<i>Saprolegnia diclina</i>	Mycelial growth	—	⦿
	Zoospore formation	—	+
<i>Achlya racemosa</i>	Mycelial growth	±	⦿
	Zoospore formation	+	+
<i>Achlya flagellata</i>	Mycelial growth	⦿	⦿
	Zoospore formation	+	+
<i>Leptolegnia caudata</i>	Mycelial growth	+	⦿
	Zoospore formation	—	+
<i>Pythium</i> sp.	Mycelial growth	+	⦿
	Zoospore formation	+	+

これは水温が低い冬季に不活発な遊走子が湖底に沈降するためと考えられる。

Achlya racemosa は低温性の種類で^{7,8)}、出現は水温が 11° 以下の冬季に限られる。湖水に広く分布する *Saprolegnia* 属は湖底泥には非常に少なく、冬季に数回見られたにすぎない。酸素を含まない水で培養すると、*Saprolegnia* 属は *Achlya* 属や *Pythium* 属に比べて生育が阻害され、また細菌類や原生動物によって影響を受けるので、湖底泥に少ないものと考えられる⁹⁾。

溶存酸素と水生菌類

湖底泥中の水生菌類の季節的消長に底層水の酸素含有量が最も重要な要因と考えられるので、実験室において溶存酸素の影響について 2, 3 の実験を行った。

実験 1: 水道水を煮沸して酸素を追出して、溶存酸素に乏しい水を作り、約 100 ml 容の酸素ビンに入れる。このなかに水生菌類がわずかに発生したアサの実を入れ、室温で 6 日間放置し、菌糸の生長と遊走子形成を観察した (Table 2)。この場合、*Saprolegnia diclina* は多量の酸素を必要とするらしく、菌糸の生長は見られないが、*Achlya flagellata* や *Pythium* sp. は酸素の乏しい水中でも菌糸の生長が見られた。

遊走子形成も種類によって異なる。*Achlya flagellata* や *Pythium* sp. は溶存酸素量に関係なく遊走子が形成されるが、*Saprolegnia diclina*、*Leptolegnia caudata* は酸素の乏しい水中では遊走子形成は見られない。

実験 2: 水生菌類の遊走子の生存におよぼす酸素量の影響を調べるため、上述の方法で酸素の乏しい水を作り、このなかに一定量の遊走子を入れ、室温に 24 時間放置した後に生存状態を観察した (Table 3)。各種類とも酸素の充分に溶存する水中では遊走子は健全であるが、*Saprolegnia diclina*、*Achlya racemosa*、*Saprolegnia monoica*、*Achlya flagellata* の遊走子は酸素の乏しい水中では 24 時間内に死滅する。しかし、湖底泥に多い *Pythium* sp. と *Dictyuchus* sp. の遊走子はほとんど無酸素の水中でもなお生存していた。

実験 3: 抵抗芽の生存に対する酸素量の影響は、上述の方法で酸素の少ない水を作り、このなかに

Table 3. Effect of oxygen tension on survival of zoospore of aquatic fungi

O ₂ ml/l	0.41	6.30
<i>Saprolegnia monoica</i>	—	+
<i>Saprolegnia diclina</i>	—	+
<i>Achlya racemosa</i>	—	+
<i>Achlya flagellata</i>	—	+
<i>Dictyuchus</i> sp.	+	+
<i>Pythium</i> sp.	+	+

Saprolegnia monoica, *Saprolegnia diclina*, *Achlya racemosa*, *Achlya flagellata*, *Isoachlya* sp. の抵抗芽を入れ、室温で 8 日間放置した後に、抵抗芽の生存状態を観察した。その結果、各種類とも 0.41 ml/l しか酸素を含まない水中でも抵抗芽は健全で、特に *Achlya flagellata* と *Achlya racemosa* では 2 カ月後でもなお生存していた。

考 察

湖底泥の水生菌類の季節的消長は、湖底泥中での繁殖の季節的な差異によるものか、あるいは水中の遊走子の沈降、または抵抗芽や卵胞子などの量的な変化によるかは重要な問題である。

水生菌類は湖底泥において抵抗芽、卵胞子、菌糸、遊走子のいずれかで存在するわけであるが、このうち水中から沈降する遊走子はきわめて短時間で死滅するので (Table 3)、湖底泥の場合にはそれほど重要とは思われない。特に夏季停滞期に底層水の酸素が消失する湖沼では、無酸素状態にも強い抵抗性をもつ抵抗芽や卵胞子の形で存在しているように思われる。実際、採泥後直ちに菌類の分離を行なった場合と、3 カ月間室温に放置したものとで菌類の出現率がほとんど変わらないことは、一部は抵抗性の強い状態で存在していることを示している。しかし、中沼の湖心部の泥に混ざっている水生植物の茎や葉の小片を滅菌水で充分洗った後に培養すると、これらの基質上に *Pythium* sp. の菌糸が繁殖していることが観察される。この事実は、湖底泥中において水生菌類が繁殖していることを示している。

摘 要

1. 中沼において 1957 年 10 月から 1958 年 9

月までの1年間、湖底泥の水生菌類の季節的消長を観察した。

2. 水生菌類の出現頻度は季節によって異なり、1月から4月にかけて最大に、底層水の酸素が消失する5月から11月にかけて最小となる。湖底泥の水生菌類の消長と底層水の溶存酸素量との間に著しい相関が見られた。

3. 中沼の湖底泥の水生菌類は、*Pythium* sp. と *Aphanomyces* sp. を主とする6種で、各種類とも著しい季節的消長を示す。1年を通じて見られるのは *Pythium* sp. だけで、*Achlya flagellata*, *Achlya racemosa*, *Dictyuchus* sp., *Aphanomyces*

sp., *Saprolegnia* sp. は循環期および冬季停滞期にだけ見られる。

4. 湖底泥での菌類の消長を理解するために、最も重要な要因と考えられる溶存酸素について2, 3の実験を行なった。その結果、菌類の繁殖や遊走子の形成には水の酸素含有量が関係することが明らかとなり、野外での観察結果が裏づけられた。

終わりに本研究に対し指導と助言を賜った東京教育大学の印東弘玄、伊藤洋両教授、市村俊英講師ならびに東京理科大学の辰野高司教授に感謝の意を表す。また実験に多大の助力をお願いした畠山忠史氏に感謝する。

文 献

- 1) 鈴木静夫, 植雑 73: 483 (1960).
- 2) 鈴木静夫, 陸水学雑誌 (投稿中).
- 3) 西条八束・辻本昭・市村俊英・高田和男, 地理学評論 27: 69 (1954).
- 4) 鈴木静夫, 日生態会誌 10: 172 (1960).
- 5) 鈴木静夫, 日生態会誌 10: 215 (1960).
- 6) 鈴木静夫, 植物研究雑誌 (印刷中).
- 7) Coker, W. C., Saprolegniaceae 201, Univ. North Carolina Press (1923).
- 8) Forbes, E. J., Mem. Proc. Manchester Lit., Phil. Soc. 79: 1 (1935).
- 9) Lund, A., D. Kgl. Danske Vidensk. Selsk. Skrifter, Naturv. og Math. Afd., 9 Raekke 6: 1 (1934).

Summary

1. The seasonal changes of aquatic fungi were studied in the bottom mud of Lake Nakanuma from October 1957 to September 1958. The water temperature of the bottom layer varied only 3° in the course of a year. The water became anaerobic from May to December in bottom layer.

2. The aquatic fungi in the profundal bottom mud showed remarkable seasonal changes. They showed distinctly a mid-summer minimum and a mid-winter maximum. The variation of fungi had correlation with the dissolved oxygen in the water of bottom layer.

3. Six species were isolated from the bottom mud through the year. *Pythium* sp. was seen through the year with the maximum in winter. *Achlya flagellata*, *Achlya racemosa*, *Dictyuchus* sp., *Aphanomyces* sp. and *Saprolegnia* sp. were obtained during the circulation and winter stagnation periods when the bottom layer water contained the oxygen.

4. Some experiments were carried out in the laboratory on the effect of oxygen upon the fungus activity. The results were in accordance with the observations in natural lakes.

ナス科植物の接木研究 I

トマト・イヌホオズキのキメラ形成について

増 淵 法 之*

Noriyuki MASUBUCHI*: Studies on Graft Hybrids in Solanaceae I.
Chimeras from the Grafting between *Solanum nigrum*
and *Lycopersicum esculentum*.

1960 年 7 月 8 日 受付

栄養雑種の形成が可能かどうかは遺伝学上大きな問題点の一つとなっており、いまなお肯定する側と否定する側に対立している。この両者の間にあって問題をより複雑にしている存在としてキメラがある。キメラに関しては古来 *Cytisus Adami* については Baur¹⁾, Buder²⁾, *Crataegomespilus* については Meyer³⁾ の研究があり、特に人為的なキメラ形成については Winkler⁴⁻⁷⁾ による一連の研究がある。トマト (*Lycopersicum esculentum*) とイヌホオズキ (*Solanum nigrum*) による Winkler の研究は、その後 Jørgensen⁸⁾ による追試、近年では Glushchenko⁹⁾, Günther¹⁰⁾ による報告がある。これらのキメラについてもキメラを構成している組織

間に相互作用を認める立場と否定する立場があり、これらは栄養雑種を肯定する立場と否定する立場との関係に一面通ずるものがある。これらの点から接木の遺伝性におよぼす影響の有無を研究することにしたが、その一環として Winkler によるキメラ形成の追試を行なった。

材料および方法

台木としてはトマト (*L. esculentum* var. *kinnari*) を使用し、接穂はイヌホオズキ 2 種 (*Solanum nigrum*, *S. villosum*) と野生系のトマト (*L. pimpinellifolium*) の計 3 種類を材料とした。*L. e.* var. *kinnari* は複葉、果実は桃色で中型果、果



第 1 図 *L. e.* × *S. n.* から得られたキメラ個体。

A, B-1 (*Solanum Gaertnerianum*), B, B-9 (*Solanum Koelreuterianum*),
C, B-12 (*Solanum Koelreuterianum*).

* 北海道大学理学部植物学教室 Botanical Institute, Faculty of Science, Hokkaido University, Sapporo, Japan.

松浦 一・山田幸男両教授還暦記念論文

実重量は 50~200 gr であり、*S. n.* は単葉、果実は黒紫色の小果、*S. v.* は単葉、表皮上の毛茸は短毛を密につけ、果実は橙黄色で、その大きさは *S. n.* にはほぼ同じである。



第2図 キメラ個体と親植物の葉形比較
A, *L. e.* B, B-6 (*S. Koelreuterianum*),
C, B-1 (*S. Gaertnerianum*), D, *S. n.*

台木のトマトは4月上旬に北大理学部温室に播種して、本葉が7・8枚になるまで育てた。接穂にした各種材料はトマトより約3週間おくらせてやはり温室に播種した。これら材料はトマトが7・8葉のときに、本葉が5・6葉となった。3つの組み合わせでいずれも40本づつの接木を行なった。接木の方法は割り接ぎ法をもちい、台木は本葉5・6葉の上部で切断し、接穂は本葉2・3葉を残してくさび型にした。ついで後はラノリンで接木部分をふさぎ、乾燥を防いだ。活着は非常によく、各組み合わせとも全部成功をみた。ついでから約3週間成育を続けさせてのち、ついで部分で接穂を切断した。この場合、接穂の小部分がくさび型に残るように注意した。その後2週間目からカルスの形成が盛んとなり、ひきつづいて多くの不定芽を形成するにいたった。この不定芽のなかからキメラ個体を挿木して調査の対象とした。

観 察 結 果

各組み合わせとも多くの不定芽を形成したが、キメラの出現については大きな差異を示した。すなわ

ち、*S. nigrum* の場合が最も多くのキメラを形成し、*S. villosum* の場合がこれにつき、*L. pimpinellifolium* の場合には1本もキメラの形成をみなかった(第1表)。

またいずれの場合にも接穂に起原をもつ不定芽は多く出現したが、台木の方の組織に由来する不定芽は同じくトマトの台木でありながら接穂の種類によって大きな差を示した。これらの原因が接木の技術的な差によるか、接木にもちいた種の間関係によるかは明らかでない。

本実験で生じたキメラは、*S. n.*, *S. v.* のいずれの場合も葉形と葉の表面における毛茸の性質によって識別でき、2つに大別することができた。1) トマト型の複葉で毛茸はイヌホオズキ型のもの、2) 大型ではあるが単葉で毛茸もイヌホオズキ型のもの、以上2種類のキメラのうち 1) 群に属するキメラは挿木の活着も良く、その後の生育が続いたが、2) 群に属するキメラでは挿木した場合と、また台木上にそのままおいた場合も次後の生育が悪く、多くは枯死した。結局秋まで生育したのは *S. n.* と *L. e.* のキメラでは 1) 群の複葉型 4 個体 (B-6, B-9, B-12, B-15) と 2) 群の単葉型 1 個体 (B-1) のみであった。*S. v.* と *L. e.* のキメラでは複葉型が2個体と単葉型が1個体であった。今回は *S. n.* と *L. e.* からのキメラ個体について調査した結果を報告する。開花をみることができたキメラ個体と親植物である *L. e.* と *S. n.* とでいくつかの性質について調査した結果は次のとおりであった。

葉における諸性質は *S. n.*, *L. e.* の親植物のほかに台木のカルスから形成された4倍性のトマトもあったので、これも調査の対象とした。気孔の大きさ(20細胞測定の平均、150倍視野でのマイクロメーター単位)では *S. n.* が 8.4, *L. e.* が 7.7 であったのに対して、B-6, B-12 はそれぞれ 8.5, 7.6 の値を示し大差がなかったが、B-9 は 12.2 で最も大きい気孔を有し、4x トマトの 10.4 よりも大き

第1表 三つの組合せにおけるキメラの出現頻度

	台 木	接 穂	個体数	台木型	接穂型	キメラ	個体数に 対するキ メラの%
A	<i>Lycopersicum esculentum</i>	<i>Solanum villosum</i>	40	28	143	4	10.0
B	<i>Lycopersicum esculentum</i>	<i>Solanum nigrum</i>	40	34	158	9	22.5
C	<i>Lycopersicum esculentum</i>	<i>Lycopersicum pimpinellifolium</i>	40	0	136	0	0.0



第3図 キメラ個体と親植物の葉の表皮細胞比較

1. *L. esculentum* (2x), 2. *L. esculentum* (4x), 3. *S. nigrum*, 4. B-1a, 5. B-1b, 6. B-6, 7. B-9, 8. B-12, 9. B-15, 10. B-21, 11. B-34, 10 はトマトの表皮起原のもの。他はすべてイヌホオズキ起原の表皮組織である。5 は著しく未発達な気孔を示す。11 は孔辺細胞は正常であるが、他の表皮細胞の未発達を示す。×150。

かった。なお特殊なものは B-1 であり、ある部分での測定値は 8.9 を示したのに対して他の部分では 3.0 の小さい値を示した。これらの測定値の間には大きな開きがあり、これらを平均することは無意味と思われたので、両者を区別し前者を B-1a、後者を B-1b とした。気孔の数 (200 倍視野での観察数 5 回平均) は *S. n.* が 21.0, *L. e.* が 46.8 であったのに対し、B-6, B-9, B-12 はそれぞれ 28.6, 13.0, 29.6 の値を示した。B-6, B-12 は *S. n.* に近い値であるが B-9 のみは *S. n.*, *L. e.* いずれの数よりもはるかに低い値を示した。なお B-1 では B-1a 部分では 53.0, B-1b 部分では 107.2 の値を示し、非常に多くの気孔が単位面積当たり観察された。このことは第 3 図でも明かなように表皮組織の分化程度の未発達が原因と考えられる。なおこの未分化は B-1b の部分で著しく、気孔の大きさが小さいこともこのためであり、たとえばある孔辺細胞は 2 細胞に分裂したまま生長が止まり、あたかも孔辺細胞の形成初期の形態を保つ状態を示し

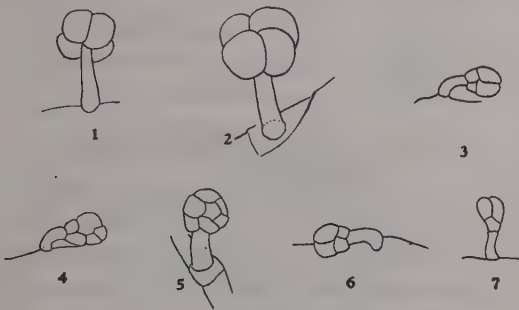
た。B-1a, B-1b の分布は葉における彎曲性と関係があり、平らに伸長した部分の表皮は B-1a の占めるところであり、裏側に彎曲した表皮部分は B-1b の占めるところであった。なお気孔の形成は十分であるが、他の表皮細胞の未発達な個体 (B-34) もあったが、この個体は開花をみずに枯死した。

毛茸の数についてみると *S. n.* が 0.4 に対して *L. e.* は 10.2 であり、単位面積当たり約 25 倍多い。これに対しキメラ個体での毛茸数は B-6, B-9, B-12, B-1a とともに 0.2 の値を示し *S. n.* よりも少ないが、この表皮系が *S. n.* 起原であることを示している。B-1b では 0.8 を示したが发育不十分のための結果と考えられる。

なおこれらキメラ個体の表皮に生じた短毛茸と *S. n.*, *L. e.* の毛茸を比較したのが第 4 図である。*S. n.* では表皮から斜めに毛茸がでており、上部の細胞は 4 つずつ 2 層になっている。これに対して *L. e.* では大型の 4 細胞からなっている。各キメラについてみると B-12 が *S. n.* に近い型を示した

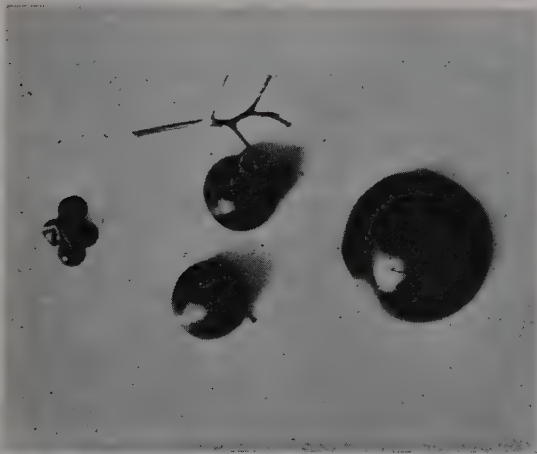
第2表 葉に見られる二、三の形質比較

	<i>S. n.</i>	<i>L. e.</i>	<i>L. e.</i> (4x)	B-6	B-9	B-12	B-1a	B-1b
気孔の大きさ	8.4±0.8	7.7±0.9	10.4±0.9	8.5±1.3	12.2±2.3	7.6±2.3	8.9±1.3	3.0±0.8
気孔の数	21.0±1.4	46.8±3.8	16.8±1.6	28.6±1.7	13.0±5.2	29.6±2.6	53.0±4.5	107.2±8.0
毛茸の数	0.4±0.5	10.2±1.5	6.2±0.8	0.2±0.4	0.2±0.4	0.2±0.4	0.2±0.4	0.8±0.8



第4図 キメラ個体と親植物の毛茸比較

1. *L. e.* (2x), 2. *L. e.* (4x), 3. *S. n.*,
4. B-6, 5. B-9, 6. B-12, 7. B-1. Ca×150



第5図 B-6の果実と親植物の果実との比較。
左 *S. n.*, 中央 B-6, 右 *L. e.*

ほか B-6, B-9 では *L. e.*, *S. n.* いずれにもない複雑な型がみられた。なお B-1 においては毛茸は表皮に直角に出ており、この点では *L. e.* に近い性質のものであった。これらの点は Glushchenko の観察結果と一致する。

花器について調べた結果は第3表のごとくであった。すなわち花卉の数は *S. n.* では一般に5であって変異がほとんどないが、*L. e.* は多弁化する傾向があり、20 花の平均で 7.6 の値を示した。同様に

キメラ個体でも多少多弁化の傾向を示し、とくに B-9 では 7.2 の値を示した。また花卉、萼片の長さは *S. n.* がそれぞれ 6.3, 2.4 mm に対して *L. e.* は 17.5, 15.4 mm であった。各キメラは *L. e.* ほどではないが、それに近いかまたは *S. n.* との中間の値を示した。なお花卉の色は *L. e.* の黄色、*S. n.* の白色に対して、これらキメラでは白色の花弁の中肋に少しく黄色を現わした点、Glushchenko, Günther の結果と同じである。そのほかキメラ個体で特徴的な点は雄ずいの葯が *L. e.* では互いに附着しているのに対してキメラでは *S. n.* 同様に分離していた。また雄ずい・雌ずいの長さも中間で、花梗のつき方も第5図に見られるように、キメラでは *L. e.* に近かった。

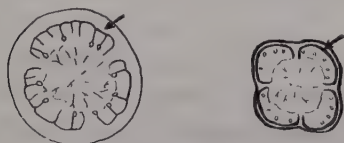
第3表 花器に見られた二、三の形質比較

	花卉の数	花卉の長さ (mm)	萼片の長さ (mm)
<i>Solanum nigrum</i>	5.0	6.3	2.4
<i>Lycopersicum esculentum</i>	7.6	17.5	15.4
B-6	6.5	15.1	8.5
B-9	7.2	15.4	8.9
B-12	5.3	14.3	7.8
B-1	6.0	15.2	5.6

果実についての調査結果は第4表のごとくであった。*S. n.* は黒紫色の果実で 20 果の平均は【0.3 gr】の小果であり、室数は2室であり変異はまれである。*L. e.* は 64 gr の平均果重を示し、室数は 3~5 室の巾を持っていた。B-1 になった果実の重量は平均 1.8 gr であり、室数は 2~4 室であった。この果実では外果皮のほか、それに隣りあった果肉部分にも黒紫色の *S. n.* に由来する色素を含み、内部の果肉部は *L. e.* の色素と思われる橙黄色の色素を有していた。また糖分含量は両植物の中間の値を示した。すなわち *L. e.* より含糖量は多かった。

第4表 果実に見られた二、三の形質比較

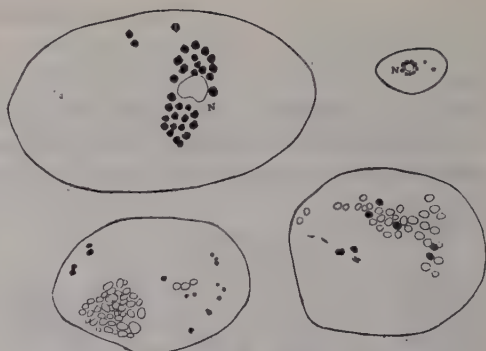
	<i>S. n.</i>	<i>L. e.</i>	B-1	B-6
果重 (gr)	0.34	63.5	1.8	10.2
室数	2	3~5	2~4	3~4
果肉細胞長さ (μ)	11.5	104.5	34.3	45.8
果肉細胞巾 (μ)	7.7	53.3	23.7	31.6
糖分含量 (%)	12.0	6.5	8.0	7.2
種子長さ (mm)	1.9	4.0	2.2	1.2
種子重量 (mg/20粒)	20	150	30	5



第6図 キメラの果実断面

た、B-6 個体の果実。室数は3で不稔種子がみえる。トマト花粉との交配による。右、B-1 個体の果実。室数は4で不稔種子を示す。自殖による。矢印は黒紫色の色素の存在部位を示す。 $\times 2/3$ 。

果肉細胞の大きさは *L. e.* が最も大きく、*S. n.* は小形であるのに対してキメラではその中間の値を示した。なお種子の形成は自殖の場合、B-1 では不稔種子のみをつけたが、*S. n.* の花粉交配では立派な種子を得た。この場合には *S. n.* の種子より多少大きい種子を得ることができた。すなわち長さでは約 15%，重さでは約 50%の増加がみられた。これに反して B-6 になった果実では次の点を特色とした。すなわち果実の色はトマトに近かったが黒味をおび、トマトの約 1/6 の大きさにすぎなかった。含糖量は約 7%で *L. e.* の含糖量に近かった。果肉細胞の大きさはやはり中間型の大きさを示した。自然の状態では結実はまだであるが、*L. e.* の花粉では良く結実した。しかし種子はすべて不稔であった。果肉細胞での著しい特色はキメラでは B-1、B-6 ともに *L. e.*、*S. n.* の細胞には見られないでんぶん粒の観察されたことであった。これら貯蔵でんぶんは糖分への転換が起こらずに残ったものと考えられるが親

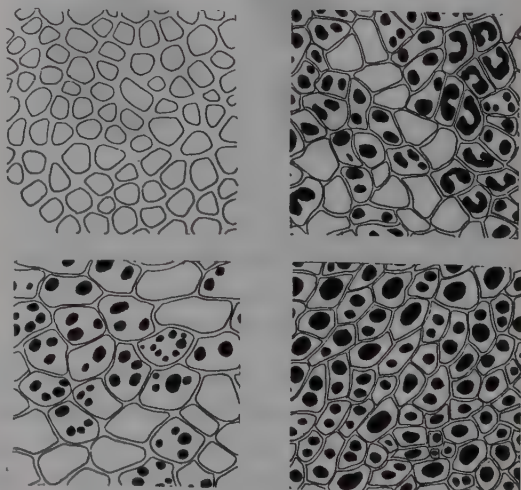
第7図 キメラ個体と親植物の果肉細胞の比較 ($\times 150$ 倍)。

左上 *L. e.*，右上 *S. n.*，左下 B-1，右下 B-6、B-1、B-6 ともにトマトに起原をもつ細胞である。黒色部は色素を、白いか粒はでんぶん粒を、また N は核を示す。

植物には見られない性質である。

果実の表皮細胞の顕微鏡観察から、このキメラではトマトとイヌホオズキの両者の色素がともに含まれていることが明らかである。なお果肉細胞では大部分はトマトのみの色素を有していたが室壁の果皮側のところはトマトの組織にもかかわらず、イヌホオズキの色素を蓄積していた。

花粉の稔性をしらべた結果は、第5表のごとくで



第8図 キメラ個体と親植物の果皮細胞の比較
左上 *L. e.*，右上 B-1，左下 B-6，右下 *S. n.*。
黒色部は紫色の色素の存在を示す。B-1 は紫色の色素を含む部分と含まぬ部分が存在することを示し、B-6 ではトマトと同じ橙黄色の色素を含む。Ca. $\times 150$ 。

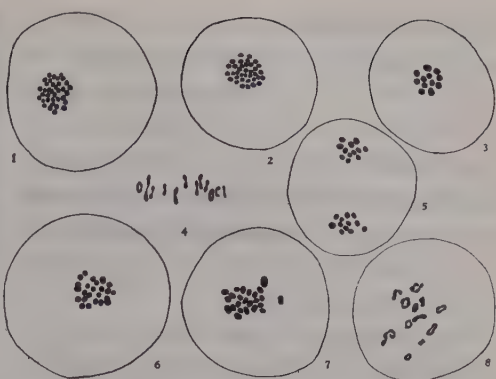
あった。 *S. n.*, *L. e.* はそれぞれ 94.1%, 93.6% の稔性を示したが、B-1, B-6, B-9, B-12 とも高い不稔性を示し、B-9 のときは約 90% の不稔であった。

第5表 花粉の稔性対

対 象 個 体	稔性%
<i>Solanum nigrum</i>	94.1
<i>Lycopersicum esculentum</i>	93.6
B-6 (<i>S. Koelreuterianum</i>)	17.8
B-9 (<i>S. Koelreuterianum</i>)	9.6
B-12 (<i>S. Koelreuterianum</i>)	12.7
B-1 (<i>S. Gaertnerianum</i>)	31.9

減数分裂の観察結果からは B-1 では $2n=72$ の染色体が観察された。大部分の分裂像は四分子形成までは正常であり、花粉不稔の原因を減数分裂の異常に帰することは困難のように思われた。それゆえ不稔の原因は四分子形成後になんらかの形で作用するように思われ今後の問題としたい。 $2n=72$ が花粉母細胞でみられたことから本植物が外層2層が *S. n.* で内部が *L. e.* の組織よりなる Winkler の *S. Gaertnerianum* と名づけられたものと考えられる。B-6, B-12 の花粉母細胞では $2n=24$ の染色体数が観察され、分裂の異常は第1, 第2分裂ともあまり見られなかった。 $2n=24$ は *L. e.* の染色体数であり、このキメラが外層1層が *S. n.* で内部が *L. e.* 組織である Winkler の *S. Koelreuterianum* である。また B-9 の花粉母細胞では $2n=48$ の染色体数が観察された。48 は *L. e.* の染色体数の倍であるとともに $(24+72)+2$ の数でもあり、特に Winkler¹¹⁾ の *S. Darwinianum* が $2n=48$ であった点から興味あるものと思われた。しかしながら第1分裂前期および diakinesis 期における観察から4価染色体の存在を確認した。このことは Kostoff¹²⁾, Lindstrom and Humphrey¹³⁾, Upcott¹⁴⁾などによるトマト4倍体の性質と同様であり、本植物が *S. Koe.* 型ではあるが、内部の *L. e.* 部分が4倍体となったものであり、Günther の場合と同様と考えられる。事実この個体から生じた芽条変異によって4倍性トマトであることが後ほど実証された。

なおこれらキメラでいま一つ特長的であったのは分枝のとき多くの枝変わりを見わしたことである。



第9図 キメラ個体と親植物の花粉母細胞第1分裂の比較

- 1. *S. n.* (IM), 2. B-1 (IM), 3. *L. e.* (IM).
- 4. B-6 (IM), 5. B-12 (IM), 6. *L. e.* 4x
- 7. B-9 (IM), 8. B-9 (diakinesis) Ca. $\times 1500$

すなわち *S. Koe.* は2個体において *S. Gae.* の枝を分枝し、*S. Gae.* の成長点は途中から *S. Koe.* に変化した。また *L. e.*, *S. n.* にそれぞれ変化した場合も数例あった。

考 察

数少ない実験であったが組み合わせた種によって異なるキメラ形成頻度を示した事実は、これらキメラの実験には材料の選択が重要であり Jörgensen の場合と同様であった。*L. e.* に *L. p.* をついだ場合に台木型が1本も形成されなかったことは接木の技術的な失敗によるものか、または接穂が台木の組織細胞の分裂を抑制する働きを有していたものであるかは不明であり、今後の問題としたい。ただ考えられることは形成されたキメラ以外に部分周縁キメラを数例生じたが、いずれも接穂型か台木型にもどり、組織間の調和が必要のように思われる。組み合わせによってはこれらの調和が保てない場合がありうるものと考えられる。

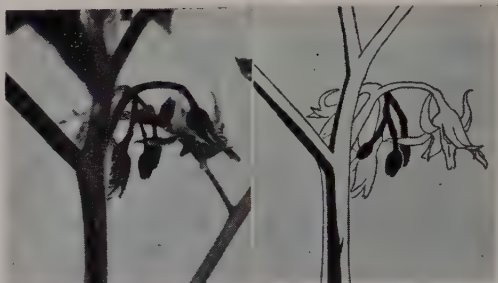
本報告で述べたキメラ個体は1層ないし2層の *S. n.* を外表とする、いわゆる周縁キメラであって、キメラ植物に見られる諸性質は親植物の中間的な形で発現しているのが多かった。これらの結果は Krenke¹⁵⁾の *L. e.* と *S. memphiticum* の場合や Glushchenko の結果と同様であった。特に表皮細胞はキメラのいずれもが *S. n.* に起原を有するものであるが、いずれも大形化し、なかでも B-9 は同じ *S. Koe.* 型であっても他の B-6, B-12 より大き

かったことは内部組織である 4x トマトの影響とみることができよう。また果実表皮細胞に B-6 ではトマトの色素を含有していた点、組織間には連絡があり相互に影響しあっているものとみられる。以上の点は Dojons¹⁶⁾のいうごとく 2つの接木成分はそれぞれ個有な形態学および生理学的な特性を保持しているものではなく、Glushchenko の主張するように相互に影響しあっている統一体とみなす見解を支持するように思われる。

S. Gae. は果実をつけるが *S. Koe.* ではほとんど結実をみなかった点 Winkler の場合と同様であった。*S. Koe.* はすべて不稔種子であったが、*S. Gae.* に属する B-1 の *S. n.* との交配種子は *S. n.* そのものの種子よりも、重量で約 50%大きかったことは、その実生の成育時においても対照より大きかったことと関連があり、一種のヘテロシス現象とみることができよう。しかし結実時期になつての差はみられなかった。

キメラ個体にみられる中間的諸形質の発現はいかように理解されるであろうか。*S. n.* と *L. e.* は実際には交配不能であり、有性交配による F_1 と比較することはできないが、花卉や萼片の多弁化、果実の室数の増加など、トマトの性質を強く現わしており、また毛茸にみられるように両者にもない複雑な形を示すことも両組織間の相互作用の結果とみなすことができよう。有性交配では両親の遺伝性が細胞内にヘテロの状態に持ちこまれ、その間に優劣の関係が作用し、いずれかの性質または中間の性質を示すが、キメラでは両者の遺伝性は細胞内にもちこまれることなしに組織別に保有されてはいるが、その関係はやはりヘテロの状態にあるといえよう。花粉や種子の稔性の悪いことは両者間の類縁関係が遠いことによる一種の雑種性不稔とみることができる。

また枝変わりが多くみられた点は成長点部位の不安定性を示すものであり、両組織の分裂能力の不均一性から細胞の組みかえが行なわれるものと考えられる。そのうち 2 例ばかりあげると次のごとくである。第 10 A 図は B-9 系統のもので、部分周縁キメラになってから引きつづいて部分的にトマトにもどったことを示し、トマトとキメラの花を 3 つずつつけたものである。第 10 B 図はそれの結実をみたところである。トマトにもどった果実はキメラの影響の有無をしらべるのに良い材料と思われたが、種



第 10 A 図 キメラからトマトに戻った花序。
黒色部は *S. n.* の表皮部分を示す。



第 10 B 図 同上果実



第 11 図 B-1 系統の果実。黒色部は
アントシアンの存在部位を
示す。Ca. $\times 2/3$.

子是不稔に終った。この不稔は本材料が 4 倍性トマトを組織として含んでいたため、キメラそのものの影響によるかは不明であった。第 11 図は B-1 系統にみられた部分周縁キメラの果実である。左は約半分が、中央は縞状に次表皮がトマトの組織に変わったものであり、この部分は紫色のアントシアンを含まない。

結 論

1) 本実験ではキメラ個体では諸形質が多くは中間型を示し、まれに両者にはない新しい形質を示すことがみられ Glushchenko の結果と一致した。

2) キメラ形成にあずかる両組織は本来の形態学的、生理学的特性をそれぞれ保持しているのではなく、相互に密接に影響しあっていることを示した。不稔花粉の増加、種子不稔の傾向はキメラの特質であったが、キメラは両組織の間における相互作用の

結果生ずる一種の雑種性のものと考えられるので、有性雑種の遠縁の場合における雑種不稔と対比させることができる。

3) 有性交配で F_1 が中間型を示し、遠縁の場合不稔となるが、この場合は受精によって生ずる細胞内ヘテロに原因するものであり、キメラの場合は異種の組織が共存することによって生ずる組織ヘテロ(細胞間)に起因する雑種と考えられる。

以上の点から Glushchenko の主張するように、キメラは雑種の一形態であり、栄養雑種の一つであると規定することができよう。

稿を終るに当たり、懇切な御指導をいただいた松浦一教授、有益な御助言を賜った明峰教授および北大理学部植物教室の方々に厚く感謝する次第である。

文 献

- 1) Baur, E., Zschr. Ind. Abst. Vererb. 1: 330 (1909).
- 2) Buder, J., Ber. Dtsch. Bot. Ges. 28: 188 (1910).
- 3) Mere, J., Zschr. Ind. Abst. Vererb. 13: 193 (1915).
- 4) Winkler, H., Ber. Dtsch. Bot. Ges. 25: 568 (1908).
- 5) —, Untersuchungen über Propfbastarde. Jena. (1912).
- 6) —, Sitz. Phys. Med. Ges. Würzburg, 95: 119 (1913).
- 7) Winkler, H., Zeit. Bot. 8: 417 (1916).
- 8) Jörgensen, C. A., and Crane, M. B., Jour. Genet. 18: 247 (1927).
- 9) Glushchenko, I. E., Vegetative hybridization in plant. Moscow. (1948).
- 10) Günther, E., Flora 144: 497 (1957).
- 11) Winkler, H., Planta 27: 680 (1938).
- 12) Kostoff, D., and Kendall, J., Gartenbauwiss. 9: 20 (1934).
- 13) Lindstrom, E. W., and Humphrey, L. M., Genet. 18: 193 (1933).
- 14) Upcott, M., Jour. Genet. 31: 1 (1935).
- 15) Krenke, N. P., Transplantation und Chimären bei Pflanzen. Berlin. (1933).
- 16) Dojons, U. N., Plant chimaeras and graft hybrids (1936).

Summary

The study on the chimeras has been carried out in several species of *Solanaceae*. The plants used as the scion are of 3 species, i.e. *S. nigrum*, *S. villosum* and *L. pimpinellifolium*, and *L. esculentum* was alone taken as the stock. Of these combinations, the case of *S. nigrum* is exclusively reported in this paper.

It was indicated from the results obtained that 1) the chimera plants showed intermediate characters in several structures, the cell size of leaf epidermis, hair styles on leaf surface, flower size, number of petals and sepals, fruit weight etc., 2) the pollen sterility of the chimeras was considerably high and the seeds in the ripe fruits from the chimera were almost defective, resembling the facts previously reported by Winkler.

It was clearly that, from the chromosome analysis, these chimeras belonged to *S. Gaer-tnerianum* and *S. Koelreuterianum* named by Winkler. Especially in one case, the $4x$ tomato-stem was surrounded by one layer of *S. nigrum*.

From these phenomena it was considered that a chimera was one of the graft hybrids. It seems to the author that these results surely support the opinion of Glushchenko.

Penicillium islandicum Sopp., NRRL 1036

の培養中における菌体色素群の消長*

菊 池 正 彦**

Masahiko KIKUCHI**: Studies on Pigment Formation in *Penicillium islandicum* Sopp., NRRL 1036 during Cultivation.

1960 年 7 月 12 日 受 付

Penicillium islandicum Sopp. は菌体色素群の構造 (Fig. 1 および前報*) Fig. 1 参照) からみると, 3 種の chemical strain に大別される^{1,2)}.

(a) 菌株 NRRL 1175: erythroskyrin, skyrin およびその同族体 oxyskyrin, pig-c などのほかに, chrysophanol などのように *para*-hydroxylation pattern をもたないアンスラキノン系色素群を生産する株.

(b) 菌株 NRRL 1036: erythroskyrin, skyrin およびその同族体などのほかに, islandicin, rubroskyrin などのように *para*-hydroxylation pattern を含むアンスラキノン系色素群を生産する株.

(c) 菌株 LSHTM BB 233: erythroskyrin だけを生産する株.

これらのうち, 著者ら^{3,4)} はさきに菌株 NRRL 1175 について培養中における色素生産の消長を調べることによって, 各色素成分の生成的関連を推定した. 本報では, 菌株 NRRL 1036 の培養中における各色素成分の消長に基づいて, それらの生成経路を考察する.

この研究にさいして, 有益な示唆と助言をいただいた柴田教授(東大薬学部)と終始御指導をいただいた林教授とに深く感謝の意を表する.

材 料 お よ び 方 法

菌株は長尾研究所から分与された *Penicillium islandicum* NRRL 1036 を用いた. 培養基の組成, 培養の方法, 色素の同定は前報³⁾と同様である.

* 前報: 本誌 73, 195-201 (1960)

** 東京教育大学理学部植物学教室 Botanical Institute, Faculty of Science, Tokyo University of Education, Ohtsuka, Tokyo, Japan.

供試菌株の色素成分について

前報³⁾と同様の方法で調べた結果, 菌株 NRRL 1036 の色素成分は Table 1 のとおりであり, 柴田ら⁵⁾の結果と一致することを確認した.

Table 1. Pigment detected in the mycelia of *P. islandicum* Sopp., NRRL 1036.

Spot	Rf	Color	Color reaction with Mg acetate	Identification
a	0.97	orange	purple	Islandicin Iridoskyrin
b	0.80	"	"	Catenarin
c	0.63	yellowish brown	yellow	Erythroskyrin
d	0.58	"	"	"
e	0.52	yellow	yellowish brown	Luteoskyrin
f	0.45	red	red	(unknown)
g	0.42	yellowish brown	orange red	Skyrin
h	0.25	brownish red	green	Rubroskyrin
i	0.19	yellowish brown	orange	Oxyskyrin (cf. Tab. 2)
j	0.05	red	greenish blue	(unknown)

なお, 構造未詳であった色素 (spot-i, Rf 0.19) はあるいは oxyskyrin ではないかと考えて, oxyskyrin の結晶標品を対照として, 供試菌株のアセトン抽出液を用いてペーパークロマトグラフ法で調べた結果, 両色素の一致が確認された (Table 2).

また, クロマトグラム上の spot-i 相当区分を溶出し, oxyskyrin の結晶標品とともに混合クロマトグラフィを行なっても, 両者の分離は見られなかった. したがって spot-i の色素を oxyskyrin とみなしてさしつかえないと考える.

Table 2. Paper chromatographic comparison of spot-i with authentic sample of oxyskyrin (Solvent: Upper layer of acetone/benzine/water 5:5:3.5, Tôyô No. 53 filter paper at 20°).

	Exp.	Rf value	Color on the chromatographic paper	Reaction with methanolic Mg acetate on the chromatogram
Spot-i obtained from the extract of the test fungus	1	0.19	yellowish brown	orange
	2	0.17		
	3	0.18		
Oxyskyrin (authentic)	1	0.19	"	"
	2	0.17		
	3	0.18		

Table 3. Sequence of pigment formation in the mycelia of *P. islandicum* Sopp., NRRL 1036 during cultivation on Czapeck-Dox solution.

Days after inoculation	Exp. 1	pH 5.8	Exp. 2	pH 6.0	Exp. 3
2	White colonies (2~3 mm. in diam.) develop.	3.6	White colonies (2~3 mm. in diam.) develop.	3.5	Eryth, Sky, Oxysky, Spot-j.
3	a) Mycelia: pale yellow. Eryth.	3.6			Eryth, Sky, Oxysky, Spot-j, Isl, Irid.
	b) Mycelia: light grayish orange. Eryth, Sky, Oxysky, Spot-j.	3.3			
	c) Mycelia: light orange-red. Eryth, Sky, Oxysky, Spot-j, Cat, Isl,) Irid.) ?	3.3			
4	Mycelia: Pinkish orange red. Eryth, Sky, Oxysky, Spot-j, Isl, Irid, Cat, Rub.	3.4~	a) Mycelia: grayish orange. Eryth, Sky, Oxysky, Spot-j, Isl, Cat.	3.3	
		3.5	b) Mycelia: grayish brown. Eryth, Sky, Oxysky, Spot-j, Isl, Cat, Rub.	3.3	
			c) Mycelia: brownish orange. Eryth, Sky, Oxysky, Spot-j, Isl, Cat, Rub, Irid.	3.3	
5	Pinkish color deepens. Spore formation occurs. Lut appears, and pigment formation is complete.	3.6			
6			Lut appears, and pigment formation is complete.	3.3	
9		4.9		4.9	Lut appears, and pigment formation is complete.

[Abbreviations] Eryth: Erythroskyrin, Sky: Skyrin, Oxysky: Oxyskyrin, Isl: Islandicin, Cat: Catenarin, Rub: Rubroskyrin, Irid: Iridoskyrin, Lut: Luteoskyrin.

Table 4. Fate of mycelial pigments after successive inoculations on the minimal media.

	Generation														Albino			
	M ₁	M ₅	M ₆	M ₇	M ₈	M ₉	M ₁₀	M ₁₁	M ₁₂	M ₁₃	M ₁₄	M ₁₅	M ₁₆	M ₁₇		M ₁₈	M ₁₉	M ₂₀
Islandicin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	±
Iridoskyrin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	±
Catenarin	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Erythroskyrin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-
Luteoskyrin	+	+	+	+	-	slightly colored	slightly colored	-	-	-	-	-	-	-	-	-	-	-
Spot-f	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Skyrin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Rubroskyrin	+	+	+	+	-*	slightly colored	slightly colored	-	-	-	-*	-*	-*	-*	-*	-	-	-
Oxyskyrin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spot-j	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-

* A brownish red spot appeared on the chromatogram, but this was distinguishable from rubroskyrin by the absence of characteristic green coloration with Mg acetate.

菌の発育過程における色素形成の順位

培養中における色素形成の順位については、すでに Shibata *et al.*³⁾ によって報告されているが、著者は、これを追試してほぼ同一の結果を得た(表 3)。

培養にさいしては *erythroskyrin* が最も早く現われるが、その生成はアンスラキノン系色素群の生成と直接的関連のないことは、さきに報告⁴⁾ したとおりである。

Skyrin, *oxyskyrin*, *spot-j* の生成順位ははっきりしなかったが、クロマトグラム上で見られるものの生成量は *skyrin* > *oxyskyrin* > *spot-j* であり培養の経過日数とともに *oxyskyrin*, *spot-j* が増量する事実からすると、*skyrin*, *oxyskyrin*, *spot-j* の順序で生成するものと推考される。

Islandicin と *iridoskyrin* とでは、*islandicin* の方が早く現われ、*rubroskyrin* と *luteoskyrin* とでは *rubroskyrin* がより早く出現する。

Catenarin の形成時期は早かったり (Table 3, Exp. 1,2) 遅かったり (Table 3, Exp. 3) して必ずしも一定しない。なお、前報³⁾ と同様に、培地の pH にはかなりの変動が見られた。

最少培地上で継代培養した場合の菌体色素の消長

前報³⁾ と同様の方法で、最少培地に継代培養を行なって、菌体色素の消長を調べた (Table 4)。

このさい、菌の所見はいわゆる *wet type* となり、色素の生産量も次第に減少する。10 代以後になると、それが特に著しく、気菌糸はきわめて少なく、かつヨジレ状となり、胞子の形成も激減する。集落は大部分がアルビノ型であり、所々に色素を生ずるものが点在し、ついにはまったく色素の生成能を失ってしまう。

Catenarin と *spot-f* とは 6 代目ころから消失し *rubroskyrin* と *luteoskyrin* は 7 代目ころからその形成能力が著しく弱まり、8 代目ではそれらの形成が見られず、9~10 代目ではわずかながら色素形成が見られたが、11 代目以後は両色素ともまったく形成されなくなった。なお、8, 14, 15, 16, 17 代目の培養ではクロマトグラム上での *rubroskyrin*

相当区分は *brownish red* を呈して *rubroskyrin* に酷似するがこのものは酢酸マグネシウムで緑色を呈しないから、別種の物質と推考される。

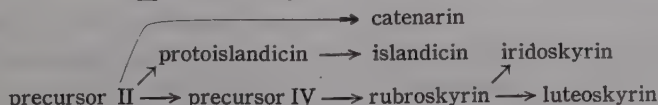
Islandicin と *iridoskyrin* とは 18 代まではともにみられ、19 代目で消失、20 代ではともに現われたがきわめて少量、21 代でははっきりせず、それ以後は完全に消失している。

Skyrin, *oxyskyrin*, *spot-j* はきわめて安定であり、他の色素が消失しても、なおかつそれらの形成はみられた。これら 3 者のうちでは、*spot-j* が他の 2 者より早く消失する。*Oxyskyrin*, *skyrin* の形成もみられなくなると菌はアルビノとなる。

考 察

柴田²⁾ は、本菌株の生産するアンスラキノン系色素群の構造上の見地から、それらの生成経路について Fig. 1 のような模式を提出している。*Acetate* から出発して、*protoflavoskyrin*, *skyrin* を経て *oxyskyrin* に至る経路を主幹とし、この経路からあふれたものが副経路を形成して *islandicin*, *rubroskyrin*, *luteoskyrin* などの形成にあずかると考えた。この *oxyskyrin* に至る主経路は、さきに報告⁴⁾ した菌株 NRRL 1175 の主経路と全く同じであり、菌株 NRRL 1036 でもそのまま当てはまると思われる。今回の色素形成の順位 (Table 3)、継代培養 (Table 4) の知見もこれを支持している。なお、構造未詳の *spot-j* は酢酸マグネシウムによる呈色反応からすればアンスラキノン系色素とみなされる。しかし、*skyrin*, *oxyskyrin*, *pig-c* の化学構造と菌株 NRRL 1175, 1036 のクロマトグラムとを比較対照すると、スポット自体の色調および酢酸マグネシウムによる呈色反応などから推して、*spot-j* は *pig-c* の誘導体 (例えば *pig-c* の 7 位または 7' 位の酸化されたもの) ではないかと考えられる。また継代培養の消長 (Table 4) からみても、*oxyskyrin* → (*pig-c*) → *spot-j* の経路が成立すると思われる。

他方、副経路については柴田は下記の生合成機構を想定し (Fig. 1 参照)、*iridoskyrin* は *luteoskyrin* あるいは *rubroskyrin* から形成されると考えた。しかし、著者が色素形成順位を調べた結果 (Table 3)



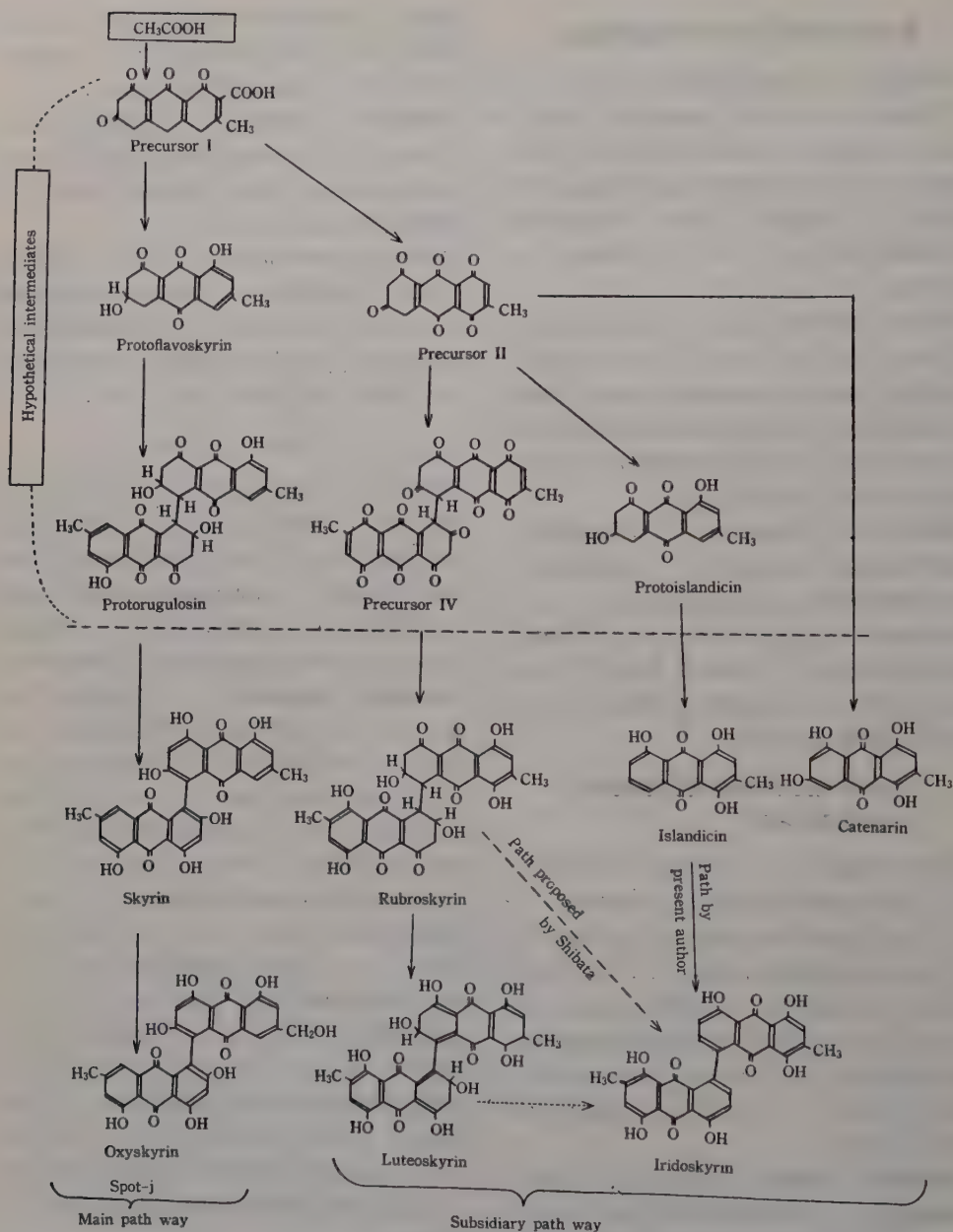


Fig. 1. Hypothetical biosynthetic scheme of pigment formation in the mycelia of *P. islandicum* Sopp., NRRL 1036.

では, luteoskyrin が最もおくれて形成されることが示されたので, luteoskyrin→iridoskyrin は考えられない。

また継代培養の結果 (Table 4) は rubroskyrin の形成がみられなくなったときでも iridoskyrin の形

成がみられることを示しているので, rubroskyrin→iridoskyrin は考えられない。ところが, iridoskyrin は islandicin よりおくれて形成され (Table 3), しかもその消長が islandicin のそれと並行する (Table 4) 事実上 islandicin→iridoskyrin の経

路を暗示している。

Rubroskyrin と luteoskyrin との関係については luteoskyrin が最もおくれて形成される (Table 3) こと, および, それらの消長が並行する (Table 4) ことなどから, rubroskyrin → luteoskyrin の経路は妥当である。

なお, 継代培養 (Table 4) の結果では islandicin, iridoskyrin が rubroskyrin, luteoskyrin よりはるかに安定なことを示しているが, このことは islandicin を経て iridoskyrin に至る経路の方が rubroskyrin を経て luteoskyrin に至る経路よりかはるかに安定なことを示すといえよう。

Catenarin の形成される時期は早かったり (Table 3, Exp. 1, 2), 遅かったり (Table 3, Exp. 3) して一定していないこと, また, 継代培養 (Table 4) で

spot-f とともに最も早く消失することなどから, この色素は生成経路の末端にあると考えられ, この点は柴田の説を支持している。

以上の実験結果から, 著者は柴田によって仮定された副経路の一部を修正して Fig. 1 で実線で示したような生合成経路を提案したい。

なお, 最近 Gatenbeck⁶⁾は, 本菌にラベルした酢酸を与えたとき, islandicin — iridoskyrin 群の specific radioactivity が rubroskyrin — luteoskyrin 群のそれよりも著しく大きいことを見だし, 菌体内では rubroskyrin や luteoskyrin から islandicin や iridoskyrin が生成することはありえないと述べている。このことは今回の著者の実験結果とも矛盾するところはない。

文 献

- 1) Howard, B.H., and Raistrick, H., Biochem. J. **57**:212 (1954).
- 2) Shibata, S., Kagaku (Japan) **26**:391 (1956).
- 3) Hayashi, K., Kikuchi, M., and Okamoto, Y., Bot. Mag. Tokyo **72**:220 (1959).
- 4) Kikuchi, M., Okamoto, Y., and Hayashi, K., ibid. **73**:195 (1960).
- 5) Shibata, S., Takido, M., and Nakajima, T., Pharm. Bull. **3**:286 (1955).
- 6) Gatenbeck, S., Communication to the 17th Intern. Congr. of Pure and Applied Chemistry, held at München on 30/8~6/9 (1959).

Summary

Based on the observations concerning the occurrence of a group of anthraquinones in the mycelia of *Penicillium islandicum* Sopp., NRRL 1036 during cultivation, the biosynthetic relationship among individual components was discussed.

The present experiments have shown that the Shibata's hypothetical biosynthetic sequence (Fig. 1) holds good only with some modifications as follows:

Iridoskyrin was always formed in the mycelia prior to the appearance of luteoskyrin (cf. Tab. 3). Iridoskyrin seems, therefore, to be derived neither from rubroskyrin nor luteoskyrin, but presumably from islandicin (Fig. 1) because of the fact that almost simultaneous appearance and disappearance could be observed between iridoskyrin and islandicin even after successive inoculation on the minimal media, whereas such a parallelism was not observed in the case of iridoskyrin and rubroskyrin.

Finally, it may be noted that the Spot-i (designated by Shibata *et al.*, 1955) was identified as oxyskyrin by paper chromatographic method (Tab. 2).

Miscellaneous Note

On the Triangular Wedge-Shaped Bamboo Piece Spawns of *Lentinus edodes* (Berk.) Sing.

by Takashi URAYAMA*

Received July 8, 1960

Triangular wedge-shaped piece spawns made of wood have been very useful for artificial cultivation of fruit bodies of *Lentinus edodes* (Berk.) Sing. However, these have two main defects: Special machine equipment is necessary for manufacture of the wooden pieces and in addition to this, preservable period of the piece spawns at normal temperature is relatively short (about 6 to 12 months). After the longer preservation the piece spawns become too soft to drive them into the logged wood for cultivation owing to decomposition of the wooden pieces.

The present study was done in order to avoid these defects. In other words, the present study was carried out to simplify the processes of the piece making and to avoid too much penetration of the mycelia into the wooden pieces.

The bamboo pieces of *Phyllostachys reticulata* C. Koch or *P. edulis* A. et C. Riv. were available for above purpose as a result of many experiments. A short bamboo piece (2 to 3 cm. in length) was prepared by splitting the bamboo cylinder through the dotted lines as shown in Fig. 1. Two kinds of the bamboo pieces obtained are shown

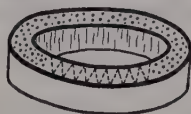


Fig. 1. Making triangular wedge-shaped bamboo pieces from short bamboo cylinder. The bamboo pieces will be made by splitting through dotted lines on the bamboo cylinder.

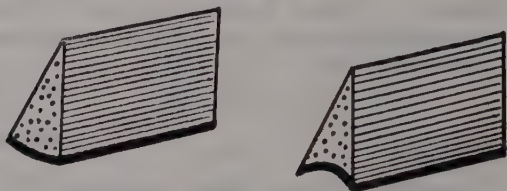


Fig. 2. Two kinds of triangular wedge-shaped bamboo pieces. Left: Piece with outer cortical layer. Right: One with inner cortical layer.

in Fig. 2. Infection of miscellaneous harmful fungi was avoided by outer or inner cortical layer (Fig. 2) of the bamboo piece which did not come in direct contact with bezel surface of the logged wood. Splitting the bamboo cylinder along the vascular bundles is easier than in the case of wood.

For production of the spawns the mycelia were inoculated on the bamboo pieces smeared with wooden sawdust and rice bran (4:1 in weight) containing a certain amount of water. An addition of sugared water (0.1%)* instead of water to the mixture resulted in the vigorous mycelial growth (Fig. 3).

The bamboo piece spawns thus made are illustrated in Fig. 4. The mycelial growth on the spawn surface and in the tissue beneath the surface was vigorous (Fig. 4), but mycelia in the inner tissue could hardly be found by microscope even after one year.

The poor mycelial growth was observed in bamboo sawdust or wooden sawdust. But with the addition of rice bran the growth was highly improved. This fact indicates

* Department of Biology, Miyazaki University, Miyazaki, Japan.

** In the case of the production of the wooden piece spawn, the addition also brought about the same result.



Fig. 3. Influence of sugared water (0.1%) upon the mycelial growth in mixture of the wedge-shaped bamboo pieces, wooden sawdust and rice bran for production of the spawns. Age, 90 days after the mycelial inoculation. Left: Control bottle with water.



Fig. 4. Triangular wedge-shaped bamboo piece spawns.

that the mycelial growth is mainly dependent upon relatively abundant nutriment in the rice bran.

Even when the amount of the mixture of the wooden sawdust and the rice bran which were mixed with the bamboo pieces was increased, promotion of the mycelial penetration into the inner tissue of the piece was not observed.

That the vigorous mycelial growth was only observed in the tissue near the surface of the bamboo might be due to that penetration of nutritious substance of the rice bran into the bamboo tissue was smaller than into the wooden one.

The bamboo piece spawn was driven into bezel which was parallel to longitudinal axis of the logged wood (Fig. 5A).

When the longitudinal axis of the spawn is vertically driven into the wood against the longitudinal axis of the wood, it is advisable to shorten the length of the piece in the direction of the vascular bundles (Figs. 2 and 5B).

The writer wishes to express his cordial thanks to Prof. Saburo Toyama and Dr. Shidai Nakayama for their encouragement and support in publishing this paper.

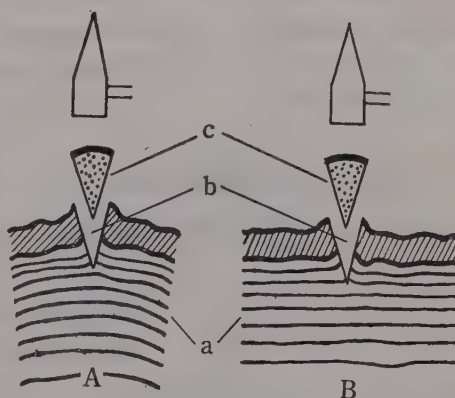


Fig. 5. Driving treatments of the bamboo piece spawns into the logged woods. A: The longitudinal axis of the spawn is parallel to that of the wood. B: The longitudinal axis of the spawn is vertical against that of the wood. a: Logged woods. b: Bezels into which the spawns are to be driven. c: Bamboo piece spawns.

摘 要 浦山隆司: シイタケの三角状楔形竹製の種駒について

シイタケ木駒を多く作るためには特別な装置を必要とし、また木駒種菌は常温で長期保存が困難である。これらの欠点を除くには竹材を用いると目的を達することができる。すなわち、輪切にして作った竹の短棒をその管束にそって縦断すれば駒の製作は容易であり、シイタケ菌糸は駒の表面と、表面近くの組織にのみ侵入生育し、さらに内部にはほとんど侵入しないので、駒種菌の長期保存が可能である。(宮崎大学学芸学部生物学教室)

植物学研究連絡委員会報告

植物学研究連絡委員会は、あたらしい委員によって 1960 年 11 月 1 日に出発した。

委員長 服部 静夫 幹事 門司 正三
 委員 芦田 譲治 服部 静夫 細川 隆英
 神谷 宜郎 木村 有香 松浦 一
 三輪 知雄 門司 正三 森 健志
 長尾 昌之 野口 弥吉 佐竹 義輔
 佐藤 敬二 下斗米直昌 和田 文吾

同日は、汎太平洋学会議 (1961 年 8 月, ハワイ) への出席希望者との連絡, 日本植物学集報

(Japanese Journal of Botany) 17 巻 3 号の編集, 外国からの分類学標本の取り扱い方につき, 学会議を通じて税関に申し出ることなどはかった。

なお, 日本植物学集報 18 巻 1 号の原稿を公募いたしますので, 集報編集委員あてに, 8 月末日までに, 原稿を書留郵便でお送りください。原稿の体裁 (とくに文献の形式) については最新号を参照してください。

日本植物学集報編集委員会

服部静夫(委員長), 原 寛, 木村有香, 前川文夫, 松浦 一, 門司正三, 大槻虎男, 田中信徳, 互理俊次 (門司正三 記)

本 会 記 事

支 部 通 信

九州支部

第 62 回例会 (12 月 10 日, 九大農学部において)
 鴨川 誠, 秋武和俊: ヤドリギの宿主選択について, 岡山繁樹: 光エネルギーが炭酸固定につかわれるまでの道すじ

れます。会長選挙の関係書類は各会員あてに 1 月中にお送りしますから, ご協力ください。

本誌巻末に会員名簿があります。この名簿に訂正すべき箇所のある方は至急お知らせください。なお住所を変更された場合は必ずお知らせください。

日本植物学会庶務幹事

会長および評議員選挙のお知らせ

昭和 36 年 2 月に会長および評議員選挙が行なわ

1961 年度植物学雑誌刊行予定

予定ページ数

1 月号	50	(他に会員名簿 20)
2 月号	40	
3 月号	70	
4 月号	40	
5 月号	70	(他に大会案内 6)
6 月号	40	
7-8 月号	80	
9 月号	60	(他に大会プログラム 16)
10 月号	40	
11-12 月号	70	(他に総索引 10)

計 560

今月は印刷のつごうにより発行がおくれましたことをおわびいたします

日本植物学会会員名簿

(支部別、アイウエオ順)

昭和 36 年 1 月 1 日現在

◎名誉会員 ○特別会員 △外国通信会員 *終身会員 括弧内は自宅住所

名誉会員	朝比奈泰彦	三宅驥一	R. R. Gates
	大木麒一	保井コノ	R. Heim
	影山藤作	羅宗洛	H. Lundegårdh
	草野俊助	J. Bonner	C. Skottsberg
	桑田義備	E. Bünning	H. St. John
	服部広太郎	R. W. Chaney	K. V. Thimann
特別会員	生駒義博	坂村徹	並河功
	石川光春	真保一輔	久内清孝
	伊藤誠哉	高嶺昇	日比野信一
	大賀一郎	武田久吉	逸見武雄
	小倉謙	多湖実輝	宮地数千木
	黒田長礼	田中長三郎	山口弥輔
	纈纈理一郎	田原正人	吉井義次
	小南清	中野治房	
外国通信会員	郑万鈞	B. Lindquist	E. H. Walker
	L. Blaringhem	J. Motte	
	F. R. Fosberg	H. Ullrich	

1. 北海道支部

秋山茂雄	北大理植 (札幌市北 28 条東 3 丁目)	岩城住江	札幌市北 16 条西 2 丁目 藤女子短大
明峯俊夫	北大理植 (札幌市南 21 条西 13 丁目)	岩淵圓	北大理植
朝比奈英三	札幌市北 8 条 北大低温科学研究所 (札幌市南 5 条西 24 丁目)	岩淵雅樹	北大理植
浅利政俊	北海道松前郡松前町字松城 144 松城小学校	宇佐美正一郎	北大理植 (札幌市北 26 条東 4 丁目)
阿部貞夫	北海道紋別市鴻之舞高校 (同市鴻之舞住吉町)	梅沢京子	北海道雨竜郡深川町 深川東高校
○伊藤誠哉	北大農植	大谷吉雄	北海道学芸大札幌分校 (北海道札幌郡琴似町筈寒 58)
伊藤太郎	帯広市外帯広畜産大生物	大野林二郎	北大理植
稲垣貫一	旭川市北門町 9 丁目 北海道学芸大旭川分校生物	大淵亮三	(函館市千代ヶ袋町 68)
井上博	札幌市琴似町川添西 琴似小学校	大山正	函館市高岡町 9 市立弥生小学校
石川鉦	北大理植 (札幌市北 13 条東 2 丁目 銀鈴荘内)	岡沢養三	北大理植 (札幌市南 17 条西 12 丁目)
猪田俊彦	網走市北 10 条西 4 丁目 網走市立第 2 中学校 (同市北 2 条西 2 丁目 鈴木五郎方)	岡本幹二	(室蘭市小橋内町 1)
入倉幸雄	北海道農事試験場作物第四研究所 (北海道千歳郡恵庭町字島松)	奥出功	北海道上川郡東川町立東川中学 (上川郡東川町西 4 号北 4 番地)
		笠原和男	北大理海藻研究所
		笠巻明子	北大理植
		加藤亮助	札幌市豊平 5 条 13 丁目 林業試験場北海道支場 (札幌市豊平 5 条 5 丁目)

- 川 嶋 昭 二 札幌市北 3 条西 5 丁目 北海道庁水産部水産課 (札幌市大通西 22 丁目)
- 川 代 善 一 北見市清見町北見営林局計画課 (同市同町)
- 川 端 清 策 岩見沢市 北海道学芸大岩見沢分校 (空知郡三笠町札幌内北区)
- 木 村 卓 郎 (札幌市南 20 条西 7 丁目)
- 北 本 毅 滝川市宇東滝川 滝川東栄高校
- 倉 林 正 尚 函館市八幡町 北海道学芸大函館分校
- 桑 山 弥 寿 男 北海道学芸大岩見沢分校
- 河 野 昭 一 北大農植 (札幌市北 7 条西 8 丁目 昭井方)
- 小 松 泰 造 芦別市 芦別市立芦別中学校
- *斎 藤 実 札幌市北海道教育委員会 学校教育課
- 斎 藤 雄 一 (札幌市北 9 条西 9 丁目)
- 酒 井 昭 札幌市北 8 条 北大低温科学研究所
- 阪 井 与 志 雄 北大理植 (札幌市北 4 条西 22 丁目)
- 佐々木 喜美子 北大理植 (札幌市南 26 条西 8 丁目)
- 佐々木 茂 釧路市大川町 48 道立水試釧路支場
- 佐々木 昭 治 北大理植 (札幌市南 26 条西 8 丁目)
- 佐々木 太 一 北海道石狩国安足間 愛山中学
- 佐藤清左衛門 北海道夕張郡栗山町 王子製紙工業材木育種研究所
- 佐 保 貴 北大理植 (札幌市南 18 条西 9 丁目 木村方)
- 沢 田 義 康 旭川市北海道学芸大旭川分校生物 (同市春光町学芸大第三職員寮)
- 渋 川 繁 光 函館市八幡町北海道学芸大函館分校生物 (函館市金堀町 136)
- 庄 貞 行 北大理植 (札幌市北 27 条西 8 丁目 荒井方)
- 田 川 隆 北大農植 (札幌市南 15 条西 5 丁目 1)
- 武 久 慎 北大理植 (札幌市北 23 条西 3 高橋方)
- 田 沢 伸 雄 北大理植 (小樽市末広町 41)
- 館 脇 操 北大農植 (札幌市北 4 条西 13 丁目)
- 田 中 滋 郎 北大理植 (札幌市南 2 条西 27 丁目 安田方)
- 田 中 瑞 穂 釧路市城山町 北海道学芸大釧路分校 (同市鶴ヶ岱 9)
- 田 辺 至 北海道留萌市千鳥町 留萌高校
- 谷 藤 茂 行 北大理植 (札幌市北 6 条西 18 丁目)
- 辻 寧 昭 北大理植 (札幌市南 23 条西 8 丁目)
- 辻 井 達 一 北大農植 (札幌市大通り西 27 丁目 小山田方)
- 寺 岡 宏 札幌市 札幌北星女子短大
- 照 本 勲 札幌市北 8 条 北大低温科学研究所 (同市北 8 条西 16 丁目)
- 時 田 郁 函館市港町 253 北大水産 (同市松蔭町 10)
- 土 橋 慶 吉 江別市宇西野幌 582 酪農学園短大図書館
- 豊 国 秀 夫 北大理植 (札幌市南 2 条西 5 丁目 31)
- 長 尾 正 人 札幌市北 27 条東 3 丁目 北大官舎
- 中 条 良 作 北海道上川郡上川町 層雲峡博物館
- 中 野 実 札幌市豊平 5 条 13 丁目 林試札幌支場豊平分場
- 中 村 義 輝 室蘭市舟見町 北大理海藻研究所
- 西 上 一 義 北大理植
- 西 田 達 郎 北海道網走郡美幌町字報徳 美幌高校 (北海道網走郡美幌町西 2 条南 3 丁目)
- 長 谷 川 由 雄 北海道余市郡余市町 北海道区水産研究所 (同町沢町 114)
- 花 房 尚 史 北大低温科学研究所
- 原 秀 雄 北大農植物園 (札幌市北 2 条西 9 丁目)
- 樋 浦 誠 北海道江別市西野幌
- 福 原 英 司 北海道余市郡余市町 北海道区水産研究所
- 舟 橋 説 往 北大理植 (札幌市宮の森 544)
- 堀 七 郎 釧路市城山町 北海道学芸大釧路分校 (同市城山町 139)
- 増 淵 法 之 北大理植 (札幌市北 8 条西 5 丁目)
- 松 浦 一 北大理植 (札幌市北 1 条西 26 丁目)
- 松 川 浩 彬 北海道学芸大函館分校生物 (函館市杉並町 200 小田桐方)
- 松 川 昌 弘 北海道虻田郡狩太町 狩太中学
- 松 坂 聰 北海道函館市八幡町 北海道学芸大函館分校生物 (北海道上磯郡上磯町字七重浜 359)
- 三 上 日 出 夫 札幌市南 18 条西 6 丁目 札幌南高校 (同市市中ノ島二区 139)
- 三 角 享 札幌市立旭ヶ丘高校
- 向 川 信 一 北大理植
- 宗 像 和 彦 函館市八幡町 北海道学芸大函館分校生物
- 森 戸 肇 北大理植

山田 幸男 北大理植 (札幌市南 11 条西 13 丁目)
 横山 竜夫 北大農植 (札幌市南 2 条西 12 丁目 山田方)
 由崎 俊道 札幌市南 22 条西 12 丁目 北海道学芸大札幌分校 (同市南 4 条西 12 丁目 三条寮)
 吉村 ふじ 北大農植 (札幌市北 14 条西 2 丁目 染野方)
 米田 一郎 北海道学芸大理植 (北海道常呂郡端野村字三区)
 和氣 和民 北大理植 (同市北 18 条西 15 丁目)
 渡会 彰彦 北大理植 (札幌市南 13 条西 10 丁目 山田方)
 渡辺 定元 帯広市東 8 条南 13 丁目 帯広営林局計画課
 (北大理 札幌市北 8 条)
 (北大農 札幌市北 8 条)

2. 東北支部

飯泉 茂 東北大理生物 (仙台市南小泉門北住宅 7 号)
 五十嵐 彰 福島県伊達郡梁川町 県立梁川高等学校 (福島県伊達郡梁川町北本町 3 坂本方)
 石塚 和雄 盛岡市上田岩手大一般教育生物
 猪狩 盛夫 東北大理生物
 伊倉 伊三美 山形市緑町 3 丁目 山形大教育生物 (同市十日町 441)
 伊藤 寛亀 宮城県栗原郡築館町 県立築館高校
 岩田 悦行 盛岡市上田岩手大学芸生物 (同大白蘭寮)
 岩淵 初郎 水沢科学博物館 (岩手県水沢市福原 16)
 上野 裕 福島県白河市字向新蔵 98
 江刺 洋司 東北大理生物 (仙台市本荒町 32)
 遠藤 冲吉 宮城県農産短大 (仙台市富沢金剛沢 16)
 遠藤 正純 東北大理生物 (仙台市五ツ谷 6-1)
 遠藤 佳孝 山形県飽海郡遊佐町菅里中学
 大森 和彦 宮城県白石市訓練場白石女子高校 (同市中河原 147-10 森方)
 大脇 頼子 東北大理生物 (仙台市北 5 番丁 15)
 奥田 慎一 東北大農芸化学
 岡根 浩造 秋田県横手市住吉町 秋田県立横手工業高校 (秋田県横手市住吉町)
 小倉 英男 東北大理生物

小田 健二 東北大理生物 (仙台市原町薬師堂北 66)
 小野 知夫 東北大川内東分校生物 (仙台市二本杉通 22)
 笠原 潤二郎 盛岡市上田 岩手大農
 榎村 利道 福島市浜田町 福島大学芸生物 (福島市桜木町 69 水広館)
 加藤 君雄 秋田市手形 秋田大学芸生物
 加藤 正名 山形市小白川町 山形大文理生物
 河原 栄治 盛岡市下厨川赤平 農林省東北農業試験場栽二
 菊池 政雄 盛岡市上田 岩手大学芸生物
 北田 宇三郎 弘前市坂本町 5 弘前学院短大
 木村 有香 東北大理生物
 ◎草野 俊助 福島県相馬市坪田
 国谷 雄三郎 群馬県館林市新宿 146
 熊野 清造 青森県弘前市弘前工業高校 (青森県弘前市笹森町 15)
 倉石 衍 東北大農芸化学 (仙台市米袋丁 27)
 栗田 精一 宮城県名取郡岩沼町字桜 47 県立名取高校 (仙台市長町字門田町 13-1)
 黒木 宗尙 塩釜市東塩釜東北海区水産研究所 (同市小松崎 61)
 小林 勝 福島市 福島大学芸生物
 小水内 長太郎 岩手県遠野市上淵町 上淵中学
 斎藤 紀 東北大川内東分校
 (釜石市栄町 2 丁目)
 佐々木 準次 秋田県鹿角郡十和田町大湯 県立十和田高校大湯分校
 佐藤 進一 弘前大理生物 (弘前市品川町 112 対島方)
 佐藤 寿子 八戸市立八戸小学校 (八戸市中居林字吹上 27)
 佐藤 久六 (青森県三戸郡五戸町大字手倉橋字荷軽井 18)
 柴岡 孝雄 東北大理生物
 島田 正雄 仙台市中島丁 7 尚綱女子学院短大 (同市土橋通 47)
 清大 大典 米沢市立米沢郷土博物館 (米沢市信濃町 2148)
 清水 芳孝 仙台市川内 東北大川内分校
 東海 林安次 秋田県大館市金板後 6 秋田県立大館鳳鳴高校
 菅谷 貞男 東北大理生物
 菅原 繁蔵 (山形県北村山郡東根市神町)
 菅原 哲二郎 (岩手県一関市山目沢内)
 杉原 美德 東北大川内東分校生物 (同市原町小田原南光沢 45-17)
 鈴木 博 仙台市川内 東北大川内分校生物 (仙台市荒巻杉添沢 1-17)
 *吹田 信英 青森市寺町

相馬寛吉 東北大理生物
 高橋信雄 山形県立新庄北高校 (山形県最上郡真室川町新町 135-4)
 高松正彦 弘前大教育野辺地分校 (青森県上北郡浦野館村大浦)
 田中清 福島市浜田町 84 福島大学芸生物
 仲尾澄子 福島市三河北町 福島県立医大薬理
 長尾昌之 東北大理生物
 中沢信午 山形市小白川町 山形大文理 (宮城県岩沼町東館下 18)
 中沢潤 弘前大文理生物
 中条幸 仙台市東3丁目 62 科学館レージャーセンター・サイエンスルーム (同市荒巻住宅東54号)
 中野敬一 青森県五所川原市 県立五所川原農林高校
 成田伝蔵 青森県五所川原市 県立五所川原高校 (同市中平井町 3)
 西崎友一郎 仙台市片平丁 41 東北大農学研究所
 沼辺征一郎 宮城県気仙沼市字町裏 66 鼎ヶ浦高校
 芳賀健一郎 (仙台市郡山字諏訪町 38)
 林義昭 東北大理生物 (仙台市角五郎丁 102)
 張尾雅信 (福島県伊達郡霊山町掛田西裏 33)
 飯田次雄 (宮城県栗原郡岩ヶ崎四番町)
 樋口利雄 (福島県飯坂局区内湯野字音ヶ森)
 日沢兼松 盛岡市北山県立雙学校 (同市北山 90)
 平井正和 青森県下北郡川内町 戸沢中学 (同県同郡大湊町川守 16)
 平田政由 弘前大文理生物 (弘前市富田字桔梗野 185-70)
 平松計之助 山形市小白川町 山形大文理
 藤田光 盛岡市上田 日本専売公社盛岡たばこ試験場
 藤原彰夫 東北大農
 三浦竹治郎 秋田県秋田市泉 秋田県農業試験場 (秋田市外旭川八柳)
 三田畔吾 岩手県稗貫郡石鳥谷町関口
 三井英二 東北大理生物 (仙台市長町越路 7-8 郷内方)
 南一守 福島市三河北町 1 福島医大細菌 (同市泉早稲田 17)
 茂木允彦 仙台市川内 東北大川内分校生物 (仙台市北五十人町 52-3 瀬戸方)
 森邦彦 鶴岡市新屋敷町 14 山形大農

安本広静 (盛岡市内丸下ノ橋際 49)
 山岸光尙 新潟県高田局区内 新潟大高田分校
 ○山口弥輔 (茨城県稲敷郡東村福田)
 山本光男 山形市小白川町 山形大文理
 結城嘉美 山形市教育委員会 (山形市神明町 1760)
 横尾弥平 山形県立東根高校 (山形県北村山郡東根町東根甲 250)
 吉岡邦二 東北大理生物
 和田俊司 東北大理生物
 (東北大理 仙台市片平丁, 東北大教育 仙)
 (台市北7番丁, 弘前大文理 弘前市富田町)

3. 関東支部

会沢正義 横浜市磯子区杉田町 1712 横浜国立大学立浜中学 (横浜市西区平沼町 3-125)
 新井澄 長野県諏訪郡原村 原中学
 相見霊三 北区西ヶ原 農技研
 青木和子 横浜国立大学芸 (川崎市新城 302)
 青山俊吉 栃木県足柄市有楽町 足柄女子高校
 阿久津彰 茨城県那珂湊市 那珂湊中学
 浅井康宏 東京歯科大保存学教室 (世田ヶ区玉川中町 1-17)
 浅野明 横須賀市浦郷町 2-32
 浅野一男 長野県下伊那郡高森町立高森北小学校
 浅野貞夫 千葉県鴨川町 長狭高校
 浅野正義 (文京区本郷 5-5)
 浅利喬泰 世田ヶ区谷区世田ヶ谷 4丁目 東京農大農芸化学
 ◎朝比奈泰彦 (新宿区戸塚町 3-123)
 足立義夫 (茨城県竜ヶ崎市大徳町 2446)
 阿部定夫 平塚市中原 農林省農業技術研究所園芸部
 阿部幸穎 日大三島高校 (栃木県足利市多田木町 570)
 荒井清司 (長野県中野市松川県住 10)
 新井養老 (文京区白山前町 1)
 新崎盛敏 東大農水産植物
 荒野久雄 世田ヶ区弦巻町 3丁目 昭和薬科大生物 (埼玉県北足立郡足立町志木 1833-2)
 有藤寛一郎 東京教育大理植 (埼玉県鳩ヶ谷町 公団住宅西国地 15号 4)
 有安勉 埼玉県入間郡坂戸町 東京教育大附属坂戸高校
 有賀裕勝 東大理植

- 安藤愛次 山梨県富士吉田市上吉田町 山梨県林業試験場
- 飯島衛 早大理工
- 幾瀬マサ 千葉県習志野市 東邦大薬
- 井口昌一郎 茨城大文理生物 (水戸市石川町 西並木住宅 19号)
- 井口やす (杉並区大宮町 1624)
- 池上義信 新潟県立新潟南高校 (新潟市上所島)
- 池田庸之助 文京区向隅弥生町 東大応微研第二研究室
- 石井隆文 東京教育大理植 (川崎市大島町 3-60)
- 石井稔 千代田区神田猿楽町 2-4 韓国 YMCA 内 韓星貿易商会
- 石川茂雄 東京教育大理植 (練馬区南大泉 526)
- 石川辰夫 東大理植 (練馬区南町 2-3740 坂井方)
- 石川広隆 目黒区下目黒 林試造林部
- 石川光春 荒川区日暮里町 9 開成学園高校 (豊島区千早町 2-22)
- *石川元助 杉並区西田町 2-342-9
- 石田肇 文京区茗荷谷町 56
- *伊集院兼高 港区芝三田 1-31
- 磯三知子 四谷第2中学 (東京都南多摩郡稲城町大丸 922)
- 板垣史郎 東京都町田市本町田 協和醸酵工業株式会社 東京研究所
- 市村俊英 東京教育大理植
- 一戸正憲 川崎市生田東長沢 9064 東京都水道局長沢浄水場
- 伊藤至 (千葉県施園町 19)
- 伊藤市郎 群馬県前橋市 群馬大学芸生物 (前橋市下出町 53)
- 伊藤信吾 世田ヶ谷区世田ヶ谷東京農大 (豊島区雑司ヶ谷 7-1110)
- 伊藤武 品川区豊町 3-350
- *伊藤洋 東京教育大理植 (文京区茗荷谷町 56)
- 井上式喜 神奈川県小田原市柳新田 28
- 井上祐光 横浜国立大植 (神奈川県平塚市入野 183)
- 井上久男 東京都西多摩郡五日市町 五日市高校
- 井上浩 東京教育大理植
- 井上行雄 (世田ヶ谷区世田ヶ谷 3-2457)
- 井上隆吉 埼玉大文理生物 (文京区駒込千駄木町 172)
- 猪熊泰三 東大農林
- 今井三子 横浜国立大農 (横浜市保土ヶ谷区権太坂 100)
- 今井百里江子 お茶の水大理生物 (新宿区下落合 3-1765)
- 今関六也 目黒区下目黒 林試 (品川区小山台 2-50)
- 入来義彦 信州大教育生物
- 岩城英夫 東大理植
- 岩波洋造 横浜市大文理生物
- 岩崎尙彦 都立大理生物 (大田区市野倉町 2)
- 岩田一彦 都立一橋高校 (杉並区上荻窪 1-92)
- 岩田敏 埼玉県児玉郡児玉町 児玉高校 (大里郡寄居町大字米野 28)
- 岩野俊逸 新潟県刈羽郡小国村横沢
- 岩本康三 港区芝海岸通り 東京水産大増殖科
- 稲本賢次郎 千代田区神田猿楽町 2-4 韓国 YMCA 鶏林書房
- 印東弘玄 東京教育大理植
- 植田利喜造 東京教育大理植
- 植松春雄 甲府市 山梨大学芸附属中学
- 植村誠次 目黒区下目黒 林試
- 宇治一登 横浜市磯子区岡村町 431 横浜学園高校 (横浜市金沢区金沢町 173 浅葉方)
- 牛島忠広 小金井市小金井新田 東京農工大繊維 (同市小金井 1832 大沢方)
- 薄井宏 宇都宮市峯町 宇都宮大農林
- 内田正之助 東京教育大理植 (墨田区隅田町 1-1283)
- 右手和夫 東京都大島町差木地 都立大島高校差木地分校
- 浦口直佐 港区芝功運町 30 普連土学園 (渋谷区伊達町 21 柴沼方)
- 蛸原富男 水戸市愛宕町 2-82 茨城県蚕業試験場
- 江本義数 (世田ヶ谷区祖師ヶ谷 1-1068)
- 遠藤徹 三島市谷田 国立遺伝研
- 大井次三郎 台東区上野公園 国立科学博物館 (北区栄町 5 公務員宿舍)
- 大内一彦 武蔵野市吉祥寺 成蹊高校生物
- 大賀一郎 (東京都府中市本町 9432)
- 大沢義信 (練馬区豊玉上 2-24)
- 大島康行 都立大理生物
- 大島永義 東大農植
- 大隅正子 文京区高田豊川町 18 日本女子大生物
- 太田次郎 お茶の水大理生物 (横浜市港北区篠原町 1930)

- 大谷 俊二 東京農大育種研 (豊島区駒込 1-32)
- 大西 一博 都立大理生物 (目黒区大原町 17-17 山岸方)
- 大平 正平 新潟県栃尾市金沢 栃尾高校
- 大槻 虎男 お茶の水大理生物 (練馬区谷原町 2-2469)
- 大場 達之 (世田ヶ谷区玉川奥沢 3-277)
- 大房 剛 都立大理生物 (大田区田園調布 3-5)
- 岡田 稔 目黒区上目黒 8-500 津村研究所 (杉並区松の木町 1144)
- 岡部 正義 北区稻付島下町 1539 小川香料 東京工場
- 岡安 広治 長野県岡谷市西堀 岡谷東高校
- 小川 進 都立大森高校 (大田区馬込町 2-1128)
- 萩原 玲二 神奈川大 (横浜市神奈川区栄町 3-48)
- 奥山 春季 台東区上野公園 国立科学博物館
- 奥山 英虎 東京都八丈島 八丈町立中之郷小学校 (八丈島八丈町大字中之郷)
- *小倉 千磨 東京都北多摩郡東村山町 狭山自然公園小倉理研分室
- 小倉 謙 (豊島区池袋 3-1542)
- 長 秀彦 (栃木県鹿沼市久保町 1609)
- 尾崎 富衛 新潟市立二葉中学 (新潟市西大畑町 5194)
- 小野 貞雄 長野県北佐久郡浅間町岩村田 長野県立岩村田高校
- *小野 記彦 都立大理生物 (渋谷区代々木西原町 896)
- 小野 幹雄 都立大理 牧野標本館 (板橋区上板橋 6-4973)
- 小野 寺直子 (東京都日野町宮 399)
- 貝原 友次郎 (浦和市上木崎 421)
- ◎影山 藤作 戸板女子短大 (世田ヶ谷区下代田町 26)
- 加崎 英男 都立大理生物 (世田ヶ谷区玉川等々力町 3-47)
- 香月 繁孝 (大田区上池上町 440)
- 笠 永博美 東大理植
- 笠原 基知治 (横浜市港北区師岡町 1128)
- 風間 智恵子 千代田区一番町 22-10 女子学院
- 鹿島 哲 (豊島区要町 1-41)
- 片岡 節二 (沼津市下香貫八重 104)
- 片田 実 木更津市木更津 東京水産大実習場
- 勝見 充行 三鷹市大沢国際キリスト教大生物
- 加藤 栄 東大理生化
- 金井 弘夫 東大理植
- 金井 龍二 文京区向岡弥生町 2 東大応微研第 7 研
- 金井 康彦 学習研究社 (杉並区大宮前 6-340)
- 金丸 信次郎 (横浜市鶴見区下末吉町 1009)
- 金子 善彦 (杉並区阿佐ヶ谷 1-696)
- 釜野 井正男 (宇都宮市宮原町 1711 41号 社宅 松田美鶴方)
- 川崎 次男 東京学芸大生物 (相模原市上鶴間 5667)
- 川崎 博 台東区立育英小学校 (練馬区春日町 2-2982)
- 川崎 庸三 (中野区千光前町 15)
- 川谷 豊彦 国立衛生試験所 (埼玉県春日部町粕壁 30)
- 川名 明 東京都府中市 東京農工大農造林研
- 川原 治之助 茨城県稲敷郡阿見町 茨城大農作物
- 菊池 正彦 東京教育大理植
- 北川 政夫 横浜国立大学芸 (目黒区上目黒 5-2390)
- 北沢 浅治 (群馬県吾妻郡嬬恋村西窪 150)
- 北見 健彦 新潟県佐渡郡相川町大字達者 新潟大理附属臨海実験所
- 北見 秀夫 新潟県両津市 両津高校
- 北村 玲子 (世田ヶ谷区東玉川町 158)
- 木下 広野 星薬科大 (三鷹市下連雀 305)
- 木下 三郎 (目黒区上目黒 8-683 大橋住宅 RF-36)
- 木下 哲雄 信州大文理生物 (松本市蟻ヶ崎北区 553-43)
- 木村 達明 文京区大塚町 56 東京教育大附属高校地学教室 (豊島区長崎 8-19)
- *木村 陽二郎 東大教養生物
- 木原 均 三島市谷田 国立遺伝研
- 草下 正夫 目黒区下目黒 林試造林
- 草薙 昭雄 東大理植 (横浜市戸塚区戸塚町 183)
- 久保田 金藏 川崎市立草津高校 (横浜市港北区岸根町 590)
- 久保田 義弘 (長野県東筑摩郡本城村西条)
- 倉石 晉 東大教養生物 (板橋区大和町 4) (滞米中)
- 倉田 悟 東大農林
- 栗原 春信 信州大教育生物
- 栗原 寿 (東京都三宅島三宅村)

- 栗山英雄 神奈川県平塚市 農技研
 黒岩澄雄 東大理理
 黒川 遼 新宿区百人町 資源研
 *黒沢幸子 東大理植 (板橋区常盤台 3-1)
 ○黒田長礼 (港区赤坂福吉町 1)
 小崎吉久 川崎市堀川町 580 明葉研究所 (川崎市古川町 156)
 小泉晴一 都立志村高校 (板橋区徳丸本町 156 青葉荘)
 郷正士 東大農造林
 河野道子 (渋谷区代々木町 3-15)
 小崎道雄 世田ヶ谷区世田ヶ谷 4 丁目 東京農業大 (世田ヶ谷区世田ヶ谷 5 丁目農大教員住宅 B-12)
 小島俊郎 山梨県富士吉田市上吉田町 山梨県林業試験場
 小林巖雄 東京教育大理地質鉱物 (豊島区巢鴨 6-1288)
 小林純子 資源研 (鎌倉市雪之下 581)
 *小林艶子 横浜市立大文理生物 (横須賀市浦賀荒巻 207)
 小林弘 東京教育大理植
 小林万寿男 東京学芸大 (北区西ヶ原 4-40 渋谷方)
 小林義夫 (府中市片町 5445)
 小林義雄 台東区上野公園 国立科学博物館
 駒嶺 穆 東大理植 (世田ヶ谷区弦巻町 1-141)
 ○小南清 藤沢市辻堂 5557
 小宮定志 千代田区富士見町 1-3 日本歯科大生物
 小宮山孝一 長野市 東部中学
 小山鐵夫 東大理植 (浦和市元町 2-53)
 榎藤安武 東京都北多摩郡小平町鈴木 恵泉女子学園短大
 昆野昭晨 北区西ヶ原 農技研遺伝生理
 斎藤規夫 東京教育大理植
 佐伯敏郎 東大理植
 酒井文三 都立大理生物
 酒井芳男 栃木県高久中学 (栃木県那須郡那須町高久橋本町 鈴木トシ方)
 坂本寧男 三島市谷田 国立遺伝研
 寒河江幸正 日本製紙株式会社山林課 (新宿区上落合 1-50-9)
 向坂道治 早大 (練馬区上石神井 2-814)
 桜井久一 共立薬大 (文京区小日向台町 2-43)
 桜井英博 東大理生化
 佐々木一郎 (大田区調布鷯ノ木町 231-10)
 佐々木正人 立教大教養第三研 (中野区江古田 2-61 江古田住宅 142号)
 佐宗守 神奈川県足柄上郡中井村立中井中学 (小田原市曾我源 518)
 佐竹義輔 台東区上野公園 国立科学博物館
 佐藤賢一 港区立港中学 (大田区仲蒲田 4-4-3)
 佐藤三郎 (千葉県安房郡鴨川町京田 225)
 佐藤茂 新潟市立鳴屋野中学 (新潟市下所島字向山 50-3)
 佐藤七郎 東大理植 (松戸市日暮ときわ平団地 1-31-103)
 佐藤重平 東大教養生物
 佐藤潤平 (東京都北多摩郡清瀬村下清戸 1009)
 佐藤正一 東大理植 (東京都北多摩郡東村山町久米川 113)
 佐藤昭二 世田ヶ谷区池尻町東京教育大農
 佐藤大七郎 東大農造林
 *佐藤正巳 茨城大文理生物 (水戸市西原町 6 区 3755)
 佐藤満彦 東大理植 (練馬区南町 2-3712)
 里見まさ子 (杉並区高円寺 3-265)
 佐原幸雄 北多摩郡小平町 農林省家畜衛生試験場 (同町小川 1586)
 砂山真理子 東邦大理生物 (渋谷区代々木初台 522)
 沢村正五 宇都宮市峯町 宇都宮大学芸生物
 三戸信人 千葉県習志野市大久保 千葉大腐敗研
 *篠遠喜人 三鷹市 国際基督教大教養生物
 柴岡弘郎 東大教養生物 (杉並区大宮前 2-700)
 柴田治 信州大文理生物 (松本市笹賀二子)
 柴田寛三 千葉県茂原市高師 東京農大農
 志平依久子 東大農水産植物 (中野区江古田 4-1526)
 島地威雄 世田ヶ谷区下馬 2 東京学芸大世田ヶ谷分校 (浦和市岸町 2-24)
 嶋津昭 お茶の水女子大理植
 島袋敬一 東大理植
 島野好次 (群馬県邑楽郡板倉町西岡甲 1362)
 四分一平内 埼玉県秩父市大宮1696 県立秩父高校 (埼玉県熊谷市柿沼 5)
 清水碩 東大理植 (文京区本郷 4-31)
 志村令郎 (山梨県大月市大月)
 志村英二 平塚市中原 1519 農林省農技研
 下河原五郎 (世田ヶ谷区玉川尾山町 176 堀内方)

- 下郡山正巳 東大理植 (杉並区松ノ木町 1208)
- 下間実 横浜市南区六つ川町大丸 1246 木原生物研
- 常谷幸雄 東京農大世田ヶ谷区船橋町 80
- 代谷次夫 東大理植 (大田区山王 1-2879 栗原方) (滯加中)
- 代谷康 東邦大理 (大田区山王 1-2879 栗原方) (滯加中)
- 神宮寺誠 山梨県立甲府第一高校 (甲府市塩部町 171)
- 真保一輔 (新潟市二葉町 1-5214)
- 神保忠男 (豊島区目白町 4-41)
- 末松直次 (世田ヶ谷区下代田町 88)
- 杉野孝雄 静岡県庵原郡由比町由比中学 (静岡県阿倍郡大河内村平野)
- 杉村康知 千葉県習志野市大久保 東邦大理生物
- 杉山有 川崎市久本 180 市立高津高校 (江東区北砂町 6-36)
- 鈴木健 新宿区柏木 2-600 東京薬科大生薬 (渋谷区伊達町 6 青木方)
- 鈴木貞雄 (宇都宮市戸祭町戸立入 2770)
- 鈴木静夫 新宿区船河原町 12 東京理科大薬学微生物化学 (板橋区常盤台町 411)
- 鈴木晋二 (千葉県印旛郡白井村白井 400)
- 鈴木武夫 東京教育大理植 (豊島区池袋 6-1965 酒井方)
- 鈴木昌友 茨城大文理生物 (水戸市堀町新田 932-7)
- 鈴木恕 東京教育大理植
- 鈴木泰 台東区北清島町 清島小学校 (足立区千住末広町 30)
- 涼野元 資源研 (港区芝三田松坂町 44)
- 須藤勇 目黒区三田 247 醸造科学研究所
- 須藤千春 平塚市中原 農技研生理遺伝
- 須藤俊造 中央区月島 3号地 東海区水産研究所
- *角倉邦彦 (杉並区和泉町 758-3)
- 諏訪隆元 平塚市農技研
- 関島安隆 杉並区女子美術大附属高校 (小金井市本町 1-1869 五味川方)
- 瀬戸治二 横浜国立大学芸生物 (神奈川県足柄郡山北町向原 883)
- 善如寺厚 桐生市天神町 群馬大工生物
- 仙波安芸 中央区銀座東七丁目 双葉ビル六階 パーキンスオリエンタルブックス
- 相馬研吾 東京教育大理植
- 相馬梯介 新潟大教育生物
- 曾根田正巳 世田ヶ谷区三宿町 380-21 長尾研究所
- 高田和男 (茨城県取手町井野台 2508)
- 高野克夫 東京教育大理植
- 高野均 群馬県立館林高校 (群馬県邑楽郡伊奈良村板倉 2425)
- 高橋一虎 豊島区椎名町 6-2272 椎名町小学校
- 高橋啓二 目黒区下目黒 林試防災部
- 高橋憲子 文京区高田豊川町 日本女子大家政
- *高橋基生 東大理植 (文京区雑司ヶ谷町 126)
- 高村省三 静岡薬科大 (静岡市太田町 42)
- 高見亘 (中野区鷺ノ宮 5-383)
- 高宮篤 東大理生化
- 田口亮平 上田市信州大織維裁桑 (同市三好町 1 稲垣方)
- 武井尙 葛飾区立中川中学 (川崎市菅 5091)
- *竹内正幸 東大理植
- 竹内亮 (北多摩郡田無町下向台 909)
- 武部啓 東大理植 (文京区駒込神明町 75)
- 武田久吉 (千代田区富士見町 3-1)
- *竹中要 三島市谷田 国立遺伝研
- 竹村英一 東京教育大理植
- 多湖実輝 千代田区富士見町 1-3 日本歯大生物 (杉並区上井草町 124)
- 田崎忠良 東京都北多摩郡小金井町 東京農工大織維 (葛飾区奥戸新町 12)
- 田沢康夫 新潟市 新潟大理生物 (同市関屋本村町 1-150)
- 館岡亜緒 (杉並区方南町 543) (滯加中)
- 館岡孝 (杉並区方南町 543)
- 田中喜一郎 品川区西品川 三共株式会社高峰研究所 (杉並区阿佐ヶ谷 2-602)
- 田中信徳 東大理植 (豊島区巣鴨町 6-1538)
- 田中靖子 (東京都立川市羽衣町 2-14)
- 田辺利雄 (世田ヶ谷区世田ヶ谷 4-412)
- 田原正人 (神奈川県中郡二ノ宮町 648)
- *田宮博 文京区向岡弥生町 東大応微研 (新宿区下落合 1-363)
- *田村寛 (北区赤羽町 3-1153)
- 丹治一義 (静岡県駿河町 2-11)
- 丹田誠之助 世田ヶ谷区世田ヶ谷 5丁目 東京農業大 (世田ヶ谷区世田ヶ谷 4-413 安田方)

- 千原光雄 静岡県賀茂郡下田町 東京教育大臨海実験所
- 塚本正実 (静岡県駿東郡玉穂村中畑 77)
- 土屋茂 高田市北陸農業試験場
- 土屋工 横浜市南区六つ川 木原生物研
- 土屋元 千代田区麴町中学 (東京都北多摩郡小金井町 1116 松本方)
- 土屋貢 群馬県立下仁田高校 (群馬県富岡市富岡 1058)
- 椿啓介 世田ヶ谷区三宿 380-21 長尾研究所 (新宿区下落合 4-1687)
- 津村孝平 横浜市立大 (横浜市神奈川区松ヶ丘 14)
- 津山尚 お茶の水大理生物 (文京区小日向台町 1)
- 勅使河原司郎 前橋市緑社町 群馬県蚕業試験場
- 手塚泰彦 都立大理生物
- 寺川博典 千葉県市川市国府台 1-1 東京医科歯科大国府台分校
- 遠山益 東京教育大理植 (板橋区成増町 93)
- *徳川義親 豊島区目白 4 丁目 徳川生物学研究所
- 徳田広 東大農水産植物
- 戸塚績 東大理植 (文京区駒込片町32)
- 豊田清修 (藤沢市鶴沼 2957)
- 鳥山英雄 杉並区井荻 東京女子大生物 (杉並区荻窪 3-64)
- 中沢和子 (大田区北千束 470)
- 中沢敬止 甲府市 山梨大生物 (同市久保町 28 佐藤方)
- 中路正義 (文京区竹早町107)
- 中島吾一 桐生市天神町 群馬大生物
- 中島道郎 (杉並区大宮前 6-427)
- 中谷茂 千葉県東葛飾郡我孫子町妻字原 1634 農電研究所
- 長友貞雄 国策パルプ工業株式会社附属農林科学研究所 (東京都北多摩郡国立町 193-2)
- 永野巖 埼玉県秩父郡野上町 長瀬秩父自然科学博物館 (秩父市上野町 820-1 秩父鉄道台宿所)
- 中野治房 (千葉県東葛飾郡我孫子町湖北)
- 永海秋三 横浜国立大学芸
- *中村幸四郎 (足立区南鹿浜町 23)
- 中村佐兵衛 東京教育大理植 (豊島区日出町1-152)
- 中村三次郎 (足立区千住 2-32)
- 中村輝子 東大教養生物 (世田谷区松原町 4-430 新井方)
- 中山弘美 北区下十条 1894 科研化学株式会社 (文京区第六天町 8 第六天住宅 145)
- 猶原恭爾 新宿区百人町 資源研 (埼玉県川越市大仙波富士の腰)
- 新関滋也 お茶の水大理生物
- 新関宏夫 埼玉県鴻巣市 関東東山試験場
- 新津恒良 慈恵医大 (目黒区下目黒 3-526)
- 西岡泰三 都立大理生物
- 西沢一俊 東京教育大理植 (文京区大塚窪町 24 同大官舎)
- 西田誠 千葉市小仲台町 千葉大文理生物
- 西村光雄 東大理生化 (滞米中)
- 新田毅 東大理植 (文京区森川町 103-米谷方)
- 二村坦孝 東京教育大理植 (北区上中里 1-35)
- 沼田真 千葉市小仲台町 千葉大文理生物 (同市弁天町 98)
- 根本和成 都立足立高校 (新宿区下落合 2-815)
- 野口市夫 東大理植
- 能勢保 (千葉市玄鼻町 302)
- 野田光蔵 新潟市 新潟大理生物 (同市秋葉通 2-3722-6)
- 野津良知 東大理植 (杉並区善福寺町387)
- 延原肇 千葉県習志野市 習志野高校
- 野村一郎 東京教育大理植
- 野村清 葛飾区青戸小学校 (北区田端町 271 金堀方)
- 野村克世 世田ヶ谷区下馬 東京学芸大
- 野村幸彦 品川区立東海中学 (港区霞町 23)
- 野本宣夫 茨城大文理生物
- 萩屋薫 新潟市 新潟大農
- 橋本徹 東大教養生物 (大田区女塚 2-9)
- 長谷川昇 川崎市立川崎高校 (埼玉県川口市芝神戸 2798)
- 長谷川正男 目黒区下目黒 林試 (藤沢市本町 5-1713) (オーストラリア滞在中)
- 畑野健一 東大農植
- 畑中信一 東大理植 (渋谷区代官山町12) (フィンランド滞在中)
- *服部静夫 東大理植 (渋谷区代々木本町 817)
- ◎服部広太郎 (千代田区駿河台 2-3-8)
- 羽田正義 長野市三輪 県立短大 (同市柳町県営アパート A 6-6)
- 花田毅一 東京教育大農 (世田ヶ谷区池尻町 461)

- 塙 順 都立大理生物 (渋谷区幡ヶ谷
 笹塚町 1362)
 浜 健 夫 明治学院大 (世田ヶ谷区喜多
 見町 2062)
 浜 谷 稔 夫 東大農林 (杉並区関根町 70)
 *林 孝 三 東京教育大理植
 林 俊 郎 東大教養生物
 林 弥 栄 八王子市長房町 林試浅川分室
 原 十 太 (大田区田園調布 4-107)
 原 襄 東大教養生物 (横浜市戸塚区
 矢部町矢部団地 24-205)
 *原 寛 東大理植 (目黒区上目黒 3-
 1817)
 原 口 義 人 群馬県太田市 県立太田女子高
 校
 原 沢 伊 世 夫 小金井市貫井北町 東京学芸大
 農
 原 田 一 世田ヶ谷区三宿町 昭和女子大
 ○久 内 清 孝 (大田区調布鶴ノ木町 231)
 日 高 醇 神奈川県秦野市名古木 日本専
 売公社秦野たばこ試験場
 檜 山 庫 三 (文京区雑司ヶ谷町 48)
 平 井 信 二 東大農木材材料
 平 塚 利 子 世田ヶ谷区池尻町 東京教育大
 農 (杉並区下高井戸 4-852)
 平 塚 直 秀 東京教育大農植物病理 (杉並
 区下高井戸 4-852)
 比 留 間 実 東京農大農農学 (神奈川県高座
 郡寒川町岡田 2600)
 広 井 敏 男 東大理植 (杉並区西荻窪 1-
 206 厚生園)
 広 川 豊 康 新潟市西畑町 新潟大理生物
 広 川 秀 夫 三島市国立遺伝研 (千代田区
 六番町 11-7)
 *福 島 博 横浜市立大文理生物 (豊島区
 長崎町 1-27-5)
 福田 育 二 郎 新宿区神楽坂 東京理科大生物
 福田 一 郎 杉並区井荻 3 丁目 東京女子大
 生物
 福田 泰 二 東大理植 (練馬区下石神井 2-
 1680)
 福田 八 十 楠 (港区麻布飯倉片町 32)
 福 永 公 平 (大田区入新井 3-139)
 福 本 日 陽 都下小金井市 東京農工大繊維
 藤 伊 正 東京教育大理植 (世田ヶ谷区
 新町 1-103)
 藤 岡 孟 彦 千葉市小仲台町 1731-33
 藤 沢 敬 一 東京教育大理植
 藤 田 善 彦 東大応微研 (世田ヶ谷区大原
 町)
 藤 田 路 一 東大薬生薬
 古 川 信 昭 大田区立大森第六中学 (横浜
 市鶴見区下末吉町 428)
 古 川 久 彦 林試保護部菌類研
 古 沢 潔 夫 文京区白山御殿町 東大理附属
 植物園
 古 瀬 義 (栃木県栃木市皆川城内町1864)
 古 谷 庫 造 (目黒区中目黒 3-1008)
 古 谷 雅 樹 東大理植 (大田区久原町 615)
 (滞米中)
 別 所 礼 子 小金井市東町 4-97-4
 保 泉 仁 子 横浜市神奈川区六角橋町 横浜
 市立六角橋中学
 宝 月 欣 二 都立大理生物
 星 川 清 親 (千葉県稲毛町 公団アパート
 1016)
 本 田 正 次 東大理植 (小金井市本町 5 の
 1718)
 本 間 健 一 郎 新潟県立金沢高校 (新潟県佐
 渡郡金井村大字千種)
 *前 川 文 夫 東大理植 (杉並区天沼 1-216)
 *前 田 禎 三 目黒区下目黒 林試
 増 田 染 一 郎 品川区大崎本町 3-624 三生製
 薬株式会社研究部 (渋谷区穂
 田 2-56)
 松 居 宗 俊 品川区西品川 1-888 三共株式
 会社研究部 (世田ヶ谷区新町
 1-84)
 松 浦 正 郎 小田原市板橋 33 旭丘女子高校
 松 崎 悦 三 資源研
 松 田 一 郎 新潟市関屋 新潟高校 (同市
 中山)
 松 原 益 太 (練馬区南町 2-3686)
 松 村 清 二 三島市谷田 国立遺伝研
 松 村 幸 雄 渋谷区常盤松町 実践女子学園
 (横浜市中区本牧三ノ谷 255)
 真 船 和 夫 東京学芸大 (杉並区天沼2-364)
 丸 山 晃 横浜市立大文理生物 (横浜市
 金沢区寺前町 52 秋山照雄方)
 三 浦 昭 雄 港区芝海岸通り 東京水産大増
 殖科
 三 井 旭 文京区向隅弥生町 東大応微研
 (滞米中)
 *三 井 高 修 (千代田区富士見町 1-4)
 三 井 養 蔵 東大理植 (世田ヶ谷区深沢町
 4-143)
 三 井 清 司 東京教育大植
 水 沢 政 雄 文京区大塚 56 跡見学園
 *水 島 う ら ら (東京都府中市東町 6464)

- 水 島 正 美 都立大理牧野標本館 (府中市東町 6464)
- 三 友 清 史 埼玉県立小川高校 (埼玉県大里郡寄居町大字寄居 346)
- *水 野 忠 敏 (渋谷区原宿 3-271)
- 南 沢 吉 三 郎 小金井市中町 2-151 東京農工大組織
- 宮 口 柁 子 王子中学 (足立区梅田町 1340)
- ◎三 宅 颯 一 (新宿区下落合 2-762)
- 宮 田 渡 長野県北安曇郡白馬村 白馬高校 (長野県大町市俵町 3-1)
- 宮 地 重 遠 東大応微研 (豊島区椎名町 6-2266)
- 宮 地 数 千 木 清泉女子大 (藤沢市辻堂2480)
- 深 山 尙 男 千葉市小仲台町 824 千葉県立千葉第二高校 (千葉市汐見丘町 39)
- 宮 脇 昭 横浜国立大学芸 (横浜市神奈川区新子安 24 新子安合同住宅 42)
- 三 輪 知 雄 東京教育大理植
- 村 上 悟 東京教育大理植
- 村 上 進 埼玉大文理生物 (世田ヶ谷区世田ヶ谷 2-1316)
- 村 上 毅 杉並区高円寺 2 農林省蚕糸試験場栽桑部
- 村 上 浩 北区西ヶ原農技研 (北区中十条 1-13)
- 村 松 淳 (松本市里山辺区荒町 3297)
- 萩 山 泰 一 新宿区百人町 資源研
- 百 瀬 静 男 千代田区霞ヶ関 文部省大学学術局
- 森 江 晃 三 東京教育大理植 (港区麻布飯倉町 5-46)
- 盛 永 俊 太 郎 北区西ヶ原町 農技研
- 守 屋 忠 之 埼玉県秩父市 尾田蔭中学 (同市 1913-1)
- 門 司 正 三 東大理植 (千代田区富士見町 2-6-3)
- 柳 下 登 府中市栄町 東京農工大一般教育
- 柳 沢 新 一 熊谷市大字熊谷 1201 埼玉県蚕業試験場
- 薬 師 寺 英 次 郎 千葉県習志野市東邦大理生物 (墨田区柳原町 7-2)
- ◎保 井 コ ノ お茶の水大理生物 (文京区東片町 148)
- 安 田 齊 松本市県町 信州大文理生物
- 安 村 明 (板橋区蓮根町 2-10 蓮根公団住宅 3041)
- 矢 野 孝 二 新潟県高田局区内 新潟高田分校
- 簀 邦 彦 北多摩郡清瀬町芝山 日本 BCG 製造株式会社
- 山 内 文 新宿区百人町 資源研 (同区戸塚町 2-26)
- 山 浦 篤 埼玉大 (板橋区板橋町 6-3440)
- 八 巻 敏 雄 東大教養生物
- 山 木 雅 子 北区西ヶ原 農技研
- 山 岸 高 旺 東京教育大理植
- 山 崎 敬 東大理植 (中野区鷺ノ宮 5-296-1 鷺ノ宮住宅)
- 山 崎 弥 三 郎 茨城県東海村 日本原子力研 (豊島区巢鴨 1 の 100)
- 山 崎 照 俊 立教大理生物物理
- 山 崎 典 子 (世田ヶ谷区世田ヶ谷 2-1169)
- 山 崎 義 人 平塚市 農技研
- 山 田 謙 二 (長野県中野市大字笠原)
- 山 田 保 千葉県印旛郡四街道町 千葉大教育 (千葉県君津郡中郷村牛袋 183)
- 山 田 晃 弘 東大教養生物
- 山 田 義 男 前橋市清王寺町 群馬大学芸生物
- 山 本 茂 東京教育大理植 (新宿区西大久保 3-118)
- 山 本 総 (北多摩郡清瀬町上清戸 595-1)
- *湯 浅 明 東大教養生物
- 横 浜 康 継 東京教育大理植 (千葉県印旛郡印西町木下 1500)
- 吉 田 治 千葉市 千葉大文理生物
- 吉 田 精 一 東大理植 (文京区林町 92)
- 吉 田 幸 弘 都立大理生物 (北区西ヶ原 4-36)
- *吉 田 吉 男 新潟市 新潟大理生物
- 吉 野 昭 朗 千葉県東葛飾郡我孫子町 電力中央研究所 農電研
- 米 田 芳 秋 三島市谷田 国立遺伝研
- 与 安 康 代 横浜市神奈川区沢渡 18 神奈川学園高校
- 若 林 裕 千葉県八日市場市イの 1630 千葉県立通達高校 (同県山武郡松尾町松尾 34)
- 和 田 善 三 長野市箱清水 県立長野西高校 (同市西之門 929)
- 和 田 文 吾 東大理植 (渋谷区神山町 6)
- 渡 辺 篤 (世田ヶ谷区世田ヶ谷 3-2093)
- 渡 辺 清 彦 千葉市小仲台町 千葉大文理生物 (同市松波町 97 県営アパート 101 号)
- 渡 辺 堯 二 (静岡県御殿場市永塚 59)

渡 辺 成 美 千葉市市場町 千葉大教育
 渡 辺 ま つ 技 千葉第二高校 (千葉市小仲台町 1743-1)
 渡 辺 良 象 荒川区立第四日暮里小学校 (都下北多摩郡保谷町上保谷大門 1720)
 渡 部 一 郎 (千葉県東葛飾郡我孫子町妻子宫 1634)
 亘 理 俊 次 東大理植 (市川市八幡町1-689)

東大理 文京区本富士町, 東大農 文京区向岡弥生町, 東大教養 目黒区駒場町, 東京教育大理 文京区大塚窪町, 都立大理 世田ヶ谷区深沢町, お茶の水大理 文京区大塚町, 横浜国立大学芸 鎌倉市雪ノ下, 横浜市立大 横浜市金沢区六浦町, 茨城大文理 水戸市渡里町, 信州大文理 松本市県町, 埼玉大文理 浦和市常盤町

4. 北 陸 支 部

旭 憲 義 (福井県大野郡鹿谷村西遅羽口)
 天 羽 良 司 金沢大教育
 有 馬 忠 雄 金沢大理植
 石 倉 成 行 (富山県上新川郡大沢野町笹津 794)
 井 原 正 昭 金沢大理植
 梅 鉢 百 子 (金沢市横山町 1-41 岡田方)
 大 田 弘 (富山県下新川郡入善町棚山新)
 小 野 寺 正 二 福井大学芸 (福井市春日泉町 1号)
 加 藤 毅 福井県武生市村口町 福井県立武生高校 (同県南条郡今庄町今庄)
 蟹 本 信 雄 福井市牧ノ島 藤島高校
 加 茂 昌 子 (山梨県中巨摩郡櫛形町小笠原 255)
 香 室 昭 園 福井大学芸
 河 合 功 金沢大理植
 小 林 貞 作 富山大文理生物
 小 牧 旌 (石川県七尾市小島町 1)
 斎 田 鋼 (金沢市栄町 36)
 斎 藤 寛 昭 (福井県鯖江市中野町 16-2)
 堺 恵 美 富山大文理 (富山県魚津市大町 134)
 里 見 信 生 金沢大理植
 *柴 田 万 年 金沢大理
 下 道 治 一 (福井県今立郡池田村寺谷)
 代 崎 良 丸 小松市立犬丸小学校 (石川県小松市大川町 178)
 進 野 久 五 郎 (富山市東中野 125)

瀬 嵐 哲 夫 金沢大教育生物
 高 桑 昇 (富山県東礪波郡戸出町大清水)
 高 野 喜 八 郎 富山県射水郡小杉町 県立小杉高校 (高岡市宮脇町 919)
 高 橋 節 也 新潟県高田市南城町 2-8-5 県立高田高校
 竹 内 繁 (福井県坂井郡長畝村篠岡1618)
 竹 内 民 男 (福井市加茂河原町 32-8)
 谷 口 博 之 金沢大理植
 玉 井 直 人 金沢大理植
 津 田 道 夫 金沢大理植
 寺 下 友 三 郎 松波中学 (石川県珠洲郡松波町秋吉)
 寺 島 英 志 (石川県羽咋郡志雄町寺山)
 中 沖 太 七 郎 富山大薬
 長 基 健 治 (石川県鶴来町)
 西 田 晃 二 郎 金沢大理植
 西 村 信 義 石川県立小松高校 (石川県能美郡大杉谷村瀬領)
 福 島 栄 七 富山大学芸
 古 池 博 金沢大理植 (富山県西礪波郡福光町吉江中 1099)
 堀 駿 子 (金沢市泉野町レ 3-3)
 政 谷 德 治 石川県珠洲郡飯田町 飯田高校
 *正 宗 厳 敬 金沢大理植
 三 上 寿 一 愛知県中島郡祖父江町 上牧祖父江中学 (愛知県中島郡祖父江町大字祖父江字高熊 川瀬方)
 柳 沢 勉 (新潟県西堀通七番町 1548 正福寺方)
 山 岡 正 尾 富山県三成中学 (同県中新川郡水橋町三郷中村 417)
 山 崎 格 富山県福野町 県立福野高校
 山 田 梯 二 (石川県加賀市大聖寺亀町 9)

(金沢大理 金沢市仙石町, 金沢大教育 金沢市弥生町, 金沢大薬 金沢市大手町, 福井大学芸 福井市牧島町, 富山大文理 富山市蓮町)

5. 中 部 支 部

天 野 一 義 名古屋市千種区 城山中学
 天 野 良 之 静岡大文理生物 (静岡市長谷町 13)
 飯 島 敬 達 (静岡県沼津市小諏訪 180)
 井 沢 三 生 名大理生物
 石 部 修 津市大谷町 三重県立大水产

- 市村昭二 愛知県守山市大森金城台 金城学院大生物物理研
- 板橋美智子 名古屋市東区大幸町 1-1 愛知学芸大名古屋分校 (名古屋市北区大野町 1-13 志賀住宅 27-303 号)
- 伊藤道夫 名大理生物
- 井波一雄 名古屋市立東山植物園 (名古屋市千種区都通 2-7)
- 今関英雅 愛知県安城市 名大農生物化学
- 牛山六男 岐阜大農 (岐阜市南長森東中島 森崎文栄方)
- 及川公平 津市 三重大学芸生物
- 太田行人 名大理生物
- 太田敬久 名大理生物 (愛知県守山市長栄 194)
- 大原準之助 (岡崎市桂町稻荷 22)
- 大村敏朗 (静岡市馬場町 6)
- 岡田善敏 岐阜大 (名古屋市千種区猪高町高針大廻間 4)
- *岡本尚 名大理化学
- 加藤俊明 (愛知県宝飯郡一宮村金沢)
- 加藤幸雄 名大理生物
- 神谷平 愛知学芸大生物 (愛知県安城市福釜新田 95)
- 川松重信 (三重県桑名郡長嶋村源部)
- 楠正貫 (名古屋市千種区虹ヶ丘南住宅 10-304)
- 熊谷三郎 (岡崎市鴨田町郷前)
- 熊沢正夫 名大教養生物 (名古屋市瑞穂区弥富町月見ヶ丘)
- 倉内一二 愛知県立豊橋東高校 (豊橋市牟呂町字若宮 109)
- 倉地金光 名大教養生物
- 栗田子郎 静岡県小笠郡浜岡町 県立池新田高校 (同県小笠郡菊川町堀之内 244)
- 香田寿男 岐阜県揖斐郡横蔵村 村立横蔵小学校
- 小竹章 (三重県多気郡明和村八木戸)
- 近藤静代 (愛知県豊田市寺部町 3-73)
- 近藤武夫 静岡県大浜松分校 (浜松市広沢町 200)
- 後藤正道 静岡県磐田郡佐久間町中部 436-2 佐久間高校
- 斎藤全生 磐田市見付静岡大農 (磐田市河原町 4055-3)
- 坂本允 名大理水質研
- 桜井昌 (静岡県浜松市名残町本町)
- 佐藤徳次 愛知県立名古屋西高校 (愛知県一宮市栄町 2-20)
- 沢井輝男 愛知学芸大名古屋分校生物 (名古屋市昭和区北山町 2-1 北山荘 3-32)
- 沢村保昌 津市 三重大学芸
- 志波秀雄 愛知県春日井市 名城大農遺伝育種研
- 柴田和平 (愛知県瀬戸市水無瀬町 37)
- *島村環 名大理生物
- 清水剛治 愛知県岡崎市丸山町 美川中学 (同市伊賀町字南郷中 24)
- 清水建美 上田市常入 信州大繊維生物
- 志村義雄 (静岡市大岩 343-8)
- 榛村恵夫 静岡大教育生物 (静岡県掛川市掛川 1272-2)
- 須賀瑛文 愛知県丹羽郡 丹陽中学 (名古屋市昭和区元宮町 6-17)
- 菅井道三 名大理生物
- 杉浦昌弘 名大理生物 (名古屋市千種区唐山町 1-74 加藤錠一方)
- 杉野武雄 岐阜県海津郡南濃町太田
- 杉本順一 静岡市八幡本町 5-9 杉本植物研究所
- 鈴木昇 名古屋市瑞穂区高田町 愛知女子大 (名古屋市外鳴海町宿地 92 森川方)
- 鈴木満帆 静岡大教養 (掛川市塩町)
- *鈴木米三 神奈川県伊勢原町子易 759 (留学中)
- 瀬木紀男 津市大谷町 三重大水産 (名古屋市昭和区広路町松風園 5)
- 高尾昭夫 名大理生物 (津市阿漕町 2408)
- 高木典雄 名大教養生物
- 高野泰吉 名大農園芸 (愛知県安城市新田町小山)
- 高谷修 岐阜県本巣郡糸貫村仏生寺 県立本巣高校
- 高橋千裕 名大教養生物
- 高橋久之 (静岡県藤枝市 益津 16-3)
- 高嶺昇 (名古屋市昭和区鶴羽町 3-8)
- 滝崎吉雄 (豊橋市瓦町南裏島営住宅 A16 号)
- 田中潔 名大教養生物
- 谷口森俊 三重県立大水産
- 寺尾恭平 三重県桑名高校 (岡崎市明大寺町西郷中 39)
- 土井田幸郎 三島市谷田 国立遺伝研
- 戸田英雄 浜松市広沢町 200 浜松市立高校 (浜松市初生町 167)
- 鳥居喜一 (愛知県新城町西新町)
- 内藤雅子 浜松市下池川町 155 信愛学園 (浜松市広沢町 74)
- 中島光夫 名古屋市立向陽高校 (愛知県守山市立大字上志段味 948)

- 中山包 信州大文理 (松本市清水東 1728)
- 西井久 三重県多気郡宮川村 村立荻原小学校栗谷分校
- 野呂寿 (三重県四日市市尺富田一色堺町浜)
- 橋本竹二郎 名古屋市中区白鳥八事裏山 名城大薬生薬
- 原田市太郎 名大理生物 (名古屋市千種区田代町字楠 158 楠荘 129)
- 日野精一 名古屋市中区瑞穂区田辺通 3 名古屋市中区立大生物
- 日比野信一 (名古屋市中区天白村八事音聞山 49)
- 平野力 静岡県藤枝市五十海 県立藤枝西高校 (静岡県志太郡広幡村構内)
- 藤井良平 名大理生物
- 藤田達也 静岡県掛川市掛川 1225 静岡県立掛川高校 (掛川市瓦町)
- 堀田康雄 名大理生物 (名古屋市中区西志賀町 913)
- 堀武義 岐阜市 岐阜大学芸
- 堀米和雄 長野鉄道管理局飯山車掌支区 (長野県中野市緑町)
- 前田英三 安城市名大農 (岡崎市中六名町 県営アパート D 18 号)
- 水谷善弥 愛知県立瑞穂高校 (名古屋市中村区大正町 2-50)
- 南喬二 (浜松市高町 111 杉浦方)
- 南川幸 三重県三重郡菟野町 菟野高校 (同町菟野 2902)
- 村田新一 (豊橋市松葉町 3-187)
- 森健志 名大理生物
- 森隆也 (岡崎市梅園町寺裏 6)
- 矢頭献一 三重大農 (津市広明町 85)
- 山本幸男 名大理生物 (岐阜県揖斐川町下新町)
- 横沢瑛二 名古屋市中区瑞穂区名古屋女学院高校 (同区中山町 4-14)
- 吉井義次 岐阜市外中町 岐阜大本部
- 吉田和典 名古屋市中区霞町 市立桜台高校
- 脇田晴美 (名古屋市中区瑞穂区船原町 7-36)
- 和田清美 静岡大文理生物
- 渡辺昌彦 信州大文理 (長野県大町市神栄町 2659)
- (名大理 名古屋市中区千種区, 名大教養 名古屋市中区瑞穂区, 静岡大文理 静岡市大岩)

6. 近畿支部

- 赤井重恭 京大農植物病理
- *芦田譲治 京大理植 (京都市左京区下鴨北園町 106)
- 阿部重美 阪大理生物
- 荒勝豊 神戸市東灘区甲南大生物 (神戸市東灘区本山町野寄)
- 庵原遜 大阪市立大理工
- 飯塚宗夫 京大食糧科学研究所応用遺伝研
- 生嶋功 大阪市立大理工 (大阪市生野区猪飼野中 1-23)
- 池田勝彦 堺市大仙町 大阪府立大農遺伝育種研
- 石上晃 兵庫県立洲本高校 (洲本市筑地町 洲本高校職員寮)
- 石田政弘 京大理植
- 伊藤五彦 (京都市左京区下鴨北園町 37)
- 稲垣幸弘 三重大学芸 (三重県阿山郡阿山村千貝)
- *稲野藤一郎 (大阪府箕面市大字小野原1567)
- 稲荷山資生 奈良市 奈良学芸大
- 稲葉通一 (姫路市大津区天満 1115 三輪方)
- *今堀宏三 大阪府豊中市柴原 阪大教養生物
- 今村駿一郎 京大農応用植物 (京都市左京区北白川上終町 28)
- 敵佐耕三 阪大理生物
- *岩田五郎左衛門 (兵庫県川辺郡川西町加茂)
- 岩田修造 神戸市東灘区御影町神戸大理生物 (同市灘区六甲台六甲住宅 10-506)
- 岩槻邦男 京大理植 (左京区北白川追分町 60 森忠治方)
- 植田勝巳 奈良女子大理植 (奈良市北魚屋西町)
- 上野実朗 大阪市立大理生物
- 梅崎勇 舞鶴市長浜 京大農水産 (福井県大飯郡加斗村飯盛)
- 江越千代子 (神戸市東灘区住吉町唐松)
- 大浦六郎兵衛 大阪女子大 (大阪市天王寺区茶臼山町 40)
- 大西健之 (兵庫県加西郡多加野村馬渡合 377)
- 岡本省吾 京大農林 (京都市左京区岩倉三宅町 72)
- 岡本嘉 和歌山市西浜 和歌山大学芸
- 小川房人 大阪市立大理工生物

- 小川 幸持 京大農農林生物応用植物
 沖 敏行 帝国人網株式会社
 奥田 光郎 京都市左京区吉田二本松町・京大分校生物
 奥田 正男 大阪市立大理工 (大阪市北区天神橋筋 5-5)
 奥貫 一男 阪大理生物
 奥野 春雄 京都工芸繊維大繊維植 (京都市右京区花園円成寺町 12)
 小倉 敏美 京都市東山区林下町 華頂女子高校
 小関 治男 京大農遺伝
 尾 辻 望 阪大理生物
 片山 忠男 京大農応用植物
 梶 良美 (神戸市兵庫区有野町上向山)
 柏田 豊 兵庫県城崎郡日高町 兵庫蚕業試験場
 加藤 一男 京大理植 (京都市左京区浄土寺石橋町 81)
 加藤 次郎 京大理植
 神谷 宣郎 阪大理生物 (芦屋市三条町81)
 亀谷 嘉夫 岩村高校 (岐阜県可児郡可児町東帷子 113-1)
 香山 時彦 和歌山大学芸生物
 川戸 峯子 大阪市東住吉区平野流町 大阪学芸大平野分校生物 (神戸市東灘区本山町森神岡)
 河原 晨 大阪市立大理工 (大阪市住吉区御崎町市営住宅 417 号)
 菊地 忠寿 京大理植
 岸 敏夫 和歌山県立箕島高校 (和歌山市古屋 400)
 岸本 卯一郎 阪大理生物
 北川 尚史 京大理植 (京都市左京区田中飛鳥井町 4 井上方)
 北川 昌典 (滋賀県甲賀郡水口町水口 2207-2)
 北村 四郎 京大理植
 衣川 堅二郎 京大農応用植物 (京都市北区衣笠北天神森町 44)
 木村 英二 神戸市長田区大谷町 2-13 兵庫県衛生研 (神戸市長田区菅原通 6-2 第三平和荘 222)
 木村 康一 京大 (京都市左京区銀閣寺町 65)
 木村 和義 京大農応用植物
 吉良 竜夫 大阪市立大理工 (大阪市東住吉区西今川町 6-29)
 金生 鉄雄 神戸市東灘区 神戸大教育 (兵庫県有馬郡三田町貴志 815)
 日下部 有信 京大理植 (京都市北区出雲路神楽町)
 久世 源太郎 京大理植 (京都市左京区下鴨中川原町 96)
 工藤 昭夫 兵庫県宝塚市小林 小林聖心女子高校
 熊野 茂 神戸大理生物 (神戸市須磨区行幸町 3-110)
 栗本 喬 (奈良県大和郡山市南郡立 520)
 ◎桑田 義備 (京都市左京区浄土寺石橋町11)
 桑名 誉 阪大理生物 (吹田市西之庄 517)
 河野 清 京都工芸繊維大 (京都市左京区嵯峨一本木町 1)
 古賀 正晴 (大阪府堺市緑丘南町 4-101)
 小島 辰男 大阪府立大教養生物
 小清水 卓二 奈良女子大植 (奈良市北市町 61)
 粉川 昭平 大阪市立大理工生物
 小西 通夫 京大農応用植物
 近衛 廉也 大阪市立大理工生物
 小室 英夫 京都女子大 (京都市上京区寺町通鞍馬口下 新御堂口町285)
 近藤 昭一郎 (神戸市垂水区神出町宝勢1369)
 斎藤 竜雄 大阪市天王寺区 府立高津高校
 左貝 アイ子 奈良女子大植 (大阪市城東区中本町 533)
 坂崎 信之 大阪府北河内郡交瑞町私市 大阪市大附属植物園
 佐藤 一郎 奈良学芸大生物
 佐藤 治雄 大阪市立大理生物 (大阪府八尾市久宝寺 2234-1)
 山 段 忠 京都学芸大生物 (福知山市猪崎 1121)
 *重 永道夫 奈良女子大植
 重 本 勝 京都市左京区下鴨半木 西京大農 (同市左京区松ヶ崎西桜木町 61)
 信 夫 隆 治 大阪市東住吉区平野 大阪学芸大分校 (堺市浜寺元町 6-911)
 柴田 憲助 京都市伏見区 府立桃山高校 (下京区三笠通大宮)
 清水 晃 阪大理生物 (大阪市西成区三日路町 37 白川荘内)
 清水 巖 (和歌山県有田郡金屋町糸川 188)
 清水 弘文 (和歌山県海草郡加太町 179)
 新家 浪雄 京大理植
 末松 四郎 和歌山大学芸生物
 菅 沼 孝之 奈良女子大植
 杉浦 寅之助 大阪市阿倍野区 阪大南校 (泉大津市助松 868)
 杉野 守 京大農応用植物

- 杉 山 弘 幸 京都市東山区山科御陵中内町
京都薬大 (枚方市大字渚 240
-6)
- 須之部 淑 男 (大阪府枚方市中宮第2団地 16
号 106)
- 鈴 鹿 紀 京都市東山区大宅坂辻町 日本
新薬山科薬用植物研究所
- 須 田 省 三 神戸大理工生物 (神戸市東灘区
御影町名屋 140-3)
- 瀬 戸 良 三 西宮市岡田山 神戸女子学院高
等部
- 瀬 野 悍 二 京大理工 (京都市左京区北白
川下池田町 24 坂田方)
- 高 樋 竜 一 奈良県添上郡樺本町樺本
- 高 木 虎 雄 京都府立園部高校 (京都市右
京区嵯峨小倉山堂の前町 13)
- 高 嶋 弘 子 神戸市生田区下山手通 7-96 親
和女子高校 (竜野市竜野町竜
野 40-4)
- 高 田 英 夫 大阪市立大理工生物
- 鷹 取 晟 二 豊中市柴原 阪大北校生物
- 高 橋 和 民 神戸大理工生物 (神戸市葺合区中
尾町 44)
- 田 川 隆 京大理工
- 滝 本 敦 京大農応用植物
- 竹 内 方 行 大阪府布施市小若江 近畿大附
属高校 (大阪府八尾市柏村165
-14)
- 田 沢 仁 阪大理工生物
- 多 田 一 郎 大阪府港区三条通 4 大阪税関
(寝屋川市池田府営住宅 66 号)
- 建 武 神戸大理工生物
- 建 部 民 雄 (大阪府茨木市茨木上泉町1202)
- 田 中 国 治 大阪府池田市 大阪学芸大池田
分校生物
- *田 中 長三郎 堺市大仙町 大阪府立大農
- 田 中 美 智 子 滋賀県大津市膳所殿町 346
- 谷 元 峰 男 滋賀県立膳所高校 (大津市膳
所網町 274)
- 田 村 道 夫 大阪市阿倍野区王子町 3 阪大
南校生物
- 俵 勝 也 尼崎市杭瀬今福 塩野義製薬研
究所 (尼崎市北竹村町 2-34)
- *塚 田 松 雄 大阪市立大理工生物
- 辻 英 夫 兵庫県篠山局区内 兵庫農大生
物
- 坪 由 宏 神戸大理工生物
- 寺 田 保 阪大理工生物
- 内 貴 信 夫 岐阜市長良 岐阜大学芸生物
- 中 井 啓 郎 兵庫県水上郡柏原 県立柏原高
校生物
- 永 井 進 大阪立大理工生物 (大阪 市城
東区古市北通 4 市営住宅 1 号
の 3)
- 永 井 玲 子 阪大理工
- 中 尾 佐 助 (京都市上京区紫野西野町 19)
- *中 沢 亮 治 (尼崎市塚本元町 5)
- 中 島 岩 通 阪大理工蛋白質研
- 中 平 良 一 京都市左京区下鴨半木町 京都
府立大農
- 中 西 哲 神戸市東灘区住吉町赤塚山 神
戸大教育生物
- 中 村 公 弘 大阪市此花区春日出町 364 市
立梅香中学
- 中 村 啓 一 滋賀県野洲町野洲 三共株式会
社野洲工場農業研究所 (草津市
滋川町 971)
- 中 村 威 京都学芸大理工生物 (京都市伏
見区深草藤ノ森町)
- 中 村 運 神戸市東灘区本山町 甲南大理工
生物 (同市同区同町寄手崎甲
南寮)
- 並 河 功 (京都市北区紫野町下柳町 36)
- 西 尾 康 三 関西学院 (西宮市上ヶ原 関
西学院教師住宅)
- 西 川 欧 一 兵庫県篠山局区内 兵庫農大作
物
- 西 辻 和 馬 (京都市東山区三条通白川橋東
5 束町 237)
- 西 村 公 臣 奈良女子大理工 (京都市伏見
区深草直達橋 6-280)
- 西 村 察 平 京都市上京区寺町宏小路 立命
館大理工 (京都市下京区若宮
通七条上ル)
- 西 山 市 三 京大農食糧科学研究所
- 根 来 健 一 郎 大津市観音寺町 京大臨湖実験
所
- 萩 本 宏 京大農応用植物
- 長 谷 川 勝 好 京都市左京区 京大農附属演習
林
- 畠 山 伊 佐 男 京大理工
- 秦 野 節 司 阪大理工生物
- 馬 場 三 吾 京大理工 (大阪府茨木市真砂)
- 浜 田 秀 男 兵庫県篠山局区内 兵庫農大植
- 浜 田 稔 京大農応用植物 (京都市左京
区永観堂町 33)
- 引 田 茂 大阪市天王寺区小宮町 府立夕
陽丘高校
- 肥 田 美 知 子 大阪女子大理工 (大阪 市住吉
区粉浜東ノ町 2-34)
- 西 門 義 一 西門菌類研究所 (大阪府高槻
市常盤町 66)
- 平 岩 公 男 天理市杣之内町 天理大 (天
理市若木町 12)
- 平 岡 俊 佑 京大理工

- 平 野 実 京大教養 (京都市左京区浄土寺西田町 74)
- 平 畑 規 和歌山県立理科短大生物 (和歌山県有田郡田殿村田口 368)
- 広江美之助 京大理植 (京都市左京区一來寺松原町 18)
- 広瀬弘幸 神戸大理生物 (神戸市垂水区西垂水町霞ヶ丘 1700)
- 深沢広祐 神戸大理生物 (神戸市東灘区御影町郡家字宇殿 7)
- 深瀬 嶽 和歌山市立和歌山商業高校 (和歌山市西浜 1596-1)
- 福村隆 京大理植 (奈良市細川町 24)
- 藤沢久雄 京大理植
- 藤田安二 大阪市大淀区大仁西 2 大阪工業技術試験所 (池田市方田町 338)
- 藤原悠紀雄 神戸大御影分校 (神戸市垂水区西垂水町上霞丘 1700)
- 舟根昇 日本新薬 (京都市南区吉祥院西の左洲ヶ西町 1-1)
- 逸見武雄 (京都市上京区紫竹下梅ノ木町 72)
- 堀田禎吉 京都工芸繊維大繊維
- 穂積和夫 大阪市立大理工生物
- 堀江格郎 兵庫県篠山局区内 兵庫農大生物
- 本郷次雄 滋賀大学芸生物 (滋賀県瀬田局区内南大萱 1051)
- 前川繁夫 三重県熊野市 市立木本中学 (同市井戸町松原)
- 孫福生 (三重県伊勢市豊川町 53)
- 増田芳雄 大阪市立大理工生物
- 松井佳一 日本真珠研究所 (京都市左京区北白川追分町 81)
- 松原聰 京大農農林生物 (大阪府布施市上小坂 766)
- 松村忠蔵 大阪市東淀川区加島町 962 田辺製薬大阪研究所
- 松村正義 (神戸市東灘区赤坂通 5-4-12)
- 松村義敏 神戸市生田区中山手通 6-36 頌栄短大 (同市兵庫区鈴蘭台孤谷 37 同和耕心寮)
- 松本賢三 大阪市天王寺区南河堀町 大阪学芸大生物
- 真山三賀雄 (尼崎市上食満字魚取 市営住宅 1440)
- 丸重啓二 京大農応用植 (京都市左京区北白川蔦町 11 高原荘)
- 丸山圭蔵 京大理植
- *三木茂 大阪市立大理工生物 (宝塚市山本 平井荘)
- 三木寿子 京大理植 (京都市上京区寺町通鞍馬口下ル)
- 三井早苗 (大阪市阿倍野区相生通 1-65)
- 光岡祐彦 京都市東山区山科大宅坂辻町 日本新薬
- 南浦清 大阪税関鑑査部 (奈良市法蓮北町 134-1)
- 村田源 京大理植 (京都市右京区川島樫田町 34-9)
- 村田茂三 京大理地球物理 (京都市中京区高倉通二条下ル)
- 桃谷好英 京大理植
- 森 博 (京都市上京区小川通一条下ル)
- 森祐二 阪大理生物
- 森下日出旗 大阪市立大理工
- 森本孝 京大理植 (大阪市住吉区浜口町 393)
- 森本哲郎 (京都府船井郡丹波町須知 塩尻方)
- 柳島直彦 大阪市立大理工
- 矢内正弘 神戸大理 (兵庫県姫路市八代中町 599)
- 柳進 (京都市東山区山科音羽稲芝町 35-3 栗津方)
- 山岸秀夫 京大理植
- 山崎俊彦 (京都府中郡峰山町字吉原)
- 山本武 大阪市立大理工
- 横村英一 京大理植 (京都市下京区油小路七条上ル)
- 吉川吉次 (芦屋市東山町 1936)
- 吉田啓正 神戸市須磨区若宮町 5 神戸市立水族館
- 吉田豊治 大阪府布施市小若江 近畿大薬学
- 吉田峯男 堺市北花田 阪大産業科学研 (大阪府泉北郡高石町南 1297)
- 吉原朝子 奈良女子大理植 (京都市中京区高倉夷川上ル)
- 依田恭二 大阪市立大理工生物
- 依田静子 京大理植
- 渡辺光太郎 京大農応用植物 (京都市伏見区豊後橋町 公務員伏見住宅 213 号)
- 渡辺弘三 (滋賀県甲賀郡甲南町深川)

(京大理 京都市左京区北白川, 京大農 京都市左京区北白川, 京都工芸繊維大 京都市北区大將軍坂田町, 阪大理 大阪市北区, 大阪市立大理工 大阪市北区南扇町, 奈良女子大 奈良市北魚屋西町, 神戸大理 神戸市東灘区御影町, 和歌山大学芸 和歌山市真砂町)

7. 中国四国支部

- 青 木 充 之 (鳥根県鯉川郡斐川村大字出西 2389)
- 秋 田 一 雄 光市大字光井 武田薬品工業株式会社光工場
- 秋 山 優 松江市西川津郡 島根大文理生物
- 朝 倉 勇 (広島県佐伯郡五日市町藤垂園)
- 浅 田 弘 治 広島大理工 (広島県佐伯郡五日市海老塩浜 723-1)
- 浅 田 泰 次 松山市榑味町 愛媛大農 (同市道後今市 997)
- 安 達 貞 夫 広島大理工
- 阿 部 近 一 (徳島県麻植郡小島町)
- 安 藤 久 次 広島大理工 (広島市己斐町 己斐住宅 1-2)
- 井 木 長 治 (岡山県倉敷局区内美和町1139)
- 池 庄 司 幸 江 (呉市広町津久茂)
- 生 駒 義 博 鳥取大農林学 (鳥取市寺町45)
- 伊 藤 秀 三 広島大理工
- 稻 葉 耕 三 広島大理工
- 犬 丸 慇 広島大畜産 (広島県沼隈郡金江村平田)
- 猪 野 俊 平 岡山大理生物 (岡山市津島 岡山大官舎)
- 井 上 正 道 岡山県浅口郡金光町 金光学園内
- 岩 井 賢 郎 高知県中村市右山 県立幡多農業高校
- 上 村 享 広島大理工 (広島市出汐町681)
- 内 川 勇 愛媛大教育生物 (松山市御幸町 217)
- 江 口 享 山口市後河原 山口大文理生物 (山口市大殿大路 川瀬哲三方)
- 遠 藤 信 雄 広島大理工
- 大 倉 永 治 岡山大理生物
- 大 原 貞 男 鳥取県米子市 県立米子南高校 (鳥取県西伯郡岸本町坂長町)
- 岡 喜 昭 広島大理工
- 小 倉 富 子 (香川県高松市丸ノ内 7-2)
- 岡 崎 光 良 岡山市津島 岡山大農
- 尾 形 英 二 下関市吉見町 農林省水産講習所
- 岡 畑 寛 広島大理工
- 岡 部 作 一 広島市宇和町 13 広島女子短大
- 岡 村 信 夫 (高知県高知市中秦泉寺 25-5)
- 岡 本 修 治 広島大理工
- 岡 本 香 広島大理工
- 生 塩 正 義 広島大理工
- 尾 島 正 人 (岡山県上房郡賀陽町大字竹荘)
- 小 川 清 (下関市長府町六波 706)
- 小 河 原 公 司 岡山大教育 (倉敷市阿知町482)
- 沖 永 哲 一 広島県賀茂郡西条町 広島農業短大
- 越 智 一 男 (愛媛県西条市中野甲 314-2)
- 越 智 春 美 鳥取市 鳥取大学芸生物
- 小 野 莞 爾 広島大理工
- 賀 来 輔 章 (下関市新開町白雲台 涼風荘 T 101 号)
- 笠 原 安 夫 倉敷市 大原農業研究所 (倉敷市白楽町 703)
- 勝 本 謙 下関市長府町 山口大農 (下関市長府町中の町)
- 加 藤 勇 夫 広島大教養生物
- 上 村 登 (高知県土佐市出間 2052)
- 河 合 晃 鳥取市吉方 320 全国椎茸普及会本部
- 川 上 明 敏 (鳥取県西伯郡西伯町大字福頼)
- 岸 谷 貞 治 郎 (広島県佐伯郡五日市町海老塩浜)
- 吉 川 知 之 広島大理工 (広島市千田町上田姫子方)
- 木 下 収 鳥取市吉方町 鳥取大農作物
- 木 村 勘 二 岡山大理生物 (倉敷市川西町)
- 木 村 孝 一 山口県宇部市 山口県立医大細胞化学研
- 木 村 晴 夫 (徳島市田宮町)
- 国 沢 鎮 雄 高知県長岡郡本山町 嶺北高校生物
- 栗 田 正 秀 愛媛大文理生物 (松山市古三津市営住宅 29)
- 国 分 寛 高松市幸町 121 香川大学芸生物 (高松市宮脇町 124-1 笠井方)
- 小 林 泰 子 (香川県九尾市郡家町 2449)
- 斎 藤 真 太 郎 松江市 島根大文理 (同市外中原 134)
- 坂 村 徹 広島市古田町古江 1370
- 佐々木 睦 男 鳥取大農 (鳥取市大榎町 17)
- 佐々木 好 之 広島大理工
- 沢 村 豪 偉 高知県赤岡局区内 県立城山高枝
- 沢 良 木 庄 一 高知県中村市 県立中村高校 (同市入田 3205)
- 塩 見 隆 行 山口市糸米 2539
- 重 信 陽 二 広島県三原市中的町 三原東高校
- 重 松 三 徳 伊予鉄道株式会社 北条営業所 (愛媛県北条市柳原局区内鹿峰 202)

- 下 瀬 敏 (松江市上乃木町西の原)
- 芝 元 和 朗 広島大理工 (広島市宇品神田通 12-486)
- *下 斗 米 直 昌 広島大理工
- 進 藤 公 夫 広島大理工
- 神 野 太 郎 愛媛大教育
- 鈴 木 兵 二 広島大理工 (広島市東観音町 1 県営アパート 1-12)
- 鈴 木 洋 太 郎 岡山大理生物 (倉敷市浜町703 木村行子方)
- 瀬 尾 正 三 島根県浜田市 県立浜田高校
- 瀬 川 道 治 広島大理工
- 関 太 郎 広島大理工 (広島市南千田町 1041 沖村政広方)
- 高 沖 武 広島大理工 (広島市吉島本町 1-653)
- 田 川 洋 二 広島大理工
- 高 須 謙 一 倉敷市住吉町 736 岡山大農業生物研
- 高 橋 節 高知県須崎市新荘 須崎高校新荘分教場 (高知県長岡郡本山町四区 武市方)
- 高 見 伸 治 広島大理工 (広島市吉島町 県営住宅 9-6)
- 田 草 川 春 重 松江市乃木福富町 島根農大植
- 竹 下 政 範 広島県佐伯郡吉和村 津田高校吉和分校
- 武 丸 恒 雄 岡山大理生物
- 竹 本 貞 一 郎 岡山大教育生物 (岡山市門田 283)
- 太 刀 掛 優 呉市広町塩焼北町 市立二河小学校
- 辰 野 誠 次 広島大理工 (広島県佐伯郡石内村)
- 田 中 隆 莊 広島大理工 (広島市鷹匠町)
- 谷 本 好 正 高知県中村市中村町 1303 県立中村高校
- 玉 置 秩 香川県木田郡三木町 香川農大
- 田 羅 征 伸 広島大理工 (広島市牛田町早稲田区 547)
- 築 地 正 勝 広島大理工 (広島市安芸郡船越町 1250)
- 寺 尾 茂 美 福山市沖野上町 広島大福山分校 (広島県松永市金江町)
- 寺 内 啓 作 広島大理工 (広島県呉市郷)
- 寺 崎 弥 助 広島県井口町 鈴峯女子短大生物 (広島県佐伯郡五日市町海老塩浜)
- 土 井 美 夫 (広島市牛田町早稲田 599-2)
- 土 岸 良 臣 広島市南千田町 修道学園 (広島市舟入南町 2-45)
- 富 永 保 人 広島県賀茂郡西条町 広島農業短大生物 (同郡川上村大字正力)
- 友 永 進 (山口県吉敷郡大内町下矢田 中原福一方)
- 中 潤 三 郎 香川県木田郡三木町 香川農大 (同町大字池戸 3384)
- 中 岡 鈴 行 三次市 酒河小学校青河分校 (広島県双三郡作木村下作木港)
- 中 島 秀 明 広島大理工 (広島市宇品 13-1331 永原方)
- 中 西 稔 広島大理工 (広島市皆実町 2-448)
- 中 原 清 士 岡山大理生物 (岡山市津島岡山大官舎)
- 中 村 純 (高知市小津町70 高知大官舎)
- 西 尾 隆 昌 広島大理工
- 西 沢 甫 (倉敷市美和町 1-157)
- 西 田 雄 行 島根大教育附属中学 (松江市中中原町清光院下)
- 西 原 礼 之 助 (岡山市南方 277)
- 西 林 長 朗 岡山大理生物 (岡山市津島福居 2264)
- 二 宮 覚 愛媛県東宇和郡 野村中学 (同郡宇和町岩木 2-321)
- 二 宮 増 夫 愛媛県東宇和郡豊海村 俵津小学校 (同郡宇和町岩木)
- 根ヶ山和弘 山口県宇部市上宇部 県立宇部高校
- 根 平 邦 人 広島大理工 (広島市東観音町 2-374)
- 野 村 義 弘 (愛媛県西宇和郡伊方町湊浦 987)
- 橋 詰 隼 人 鳥取市吉方 鳥取大農林 (鳥取市立川町 2 中島方)
- 橋 本 清 美 広島大理工 (広島市皆実町 3-935)
- 橋 本 静 子 広島大理工 (広島市東雲町574-3)
- 林 克 巳 広島大理工
- 原 幹 雄 高知市小津町 70 高知大文理生物
- 原 本 春 香 広島市東雲町 広島大東雲分校附属中学
- 春 山 皓 也 広島大理工
- 日 出 武 敏 鳴門市撫養町南浜 鳴門市立第一中学
- 日 野 巖 山口大農 (山口県宇部市東区笹山)
- 広 江 三 樹 三 郎 岡山県和気郡吉永局区内 和気高校閑谷校舎
- 広 本 一 由 山口県熊毛郡平生町 熊毛南高校
- 藤 井 正 治 島根県安来市 安来高校
- 藤 茂 宏 岡山大理生物

- 藤田哲夫 広島市皆実町3 広島大教養
(広島市皆実町1 RA 31)
- 藤田幹雄 愛媛県新居浜市 新居浜東高校
- 藤山和恵 福山曉の星女子学院生物 (福
山市外大津野 広島大水産学部
宿舍)
- 藤山虎也 広島大水産植物 (福山市外大
津野 広島大水産学部宿舍)
- *堀川芳雄 広島大理植
- 本田稔 広島大理植
- 益森静生 広島大理植
- 松田忠男 広島大理植 (広島市皆実町3
-787 沖田温方)
- 松村敏則 広島大理植
- 丸山巖 島根県仁多郡横田町 県立横田
高校
- 御江久夫 (山口県徳山市大字下上 687)
- 満石光明 愛媛県西宇和郡伊方町 伊方中
学
- 三原勉 松山市東長戸 640 市立鴨川中
学 (松山市西須賀町 2245-2)
- 南敦 山口県柳井市 柳井中学 (山口
県熊毛郡田布施町松尾)
- 宮崎基快 高知県安芸市伊尾木 市立伊尾
木中学 (同県香美郡夜須町西
山 319)
- 宮本義男 愛媛大文理生物
- 三好茂正 松江市西川津町 島根大生物
(島根県八束郡八雲村東岩坂
249)
- 三好教夫 広島大理植 (広島市東千田町
453 吉尾勉方)
- 村山徹郎 愛媛大文理生物
- 村山晴子 松山市城北 愛媛大文理生物
- *森千春 広島市立青崎中学 (広島県安
芸郡府中町外新開 6021)
- 森敏之 (山口市東山通古熊町9)
- 守田治夫 (山口県大島郡橘町安下庄西浦
安下庄高校住宅)
- 森本泰二 (広島県高田郡吉田町上入江)
- 柳田文雄 高知市朝倉 高知大教育生物
(同大教育学部校舎)
- 矢野悟道 広島大理植
- 山下博太郎 広島県佐伯郡五日市町 明治製
菓広島工場
- 山下昌徳 香川県小豆郡内海町 県立小豆
島高校 (内海町坂手甲 33-1)
- 山田清 宇部市立神原中学 (山口県宇
部市東区琴芝台 815-2)
- 山中二男 高知市朝倉 高知大教養生物
- 山根仁文 岡山大理生物
- 山本昌木 松江市乃木福富町 島根農大植
物病理 (松江市上乃木町西原
島根農大公舎)
- 山本四郎 松山市末広町 松山南高校
- 山本正明 広島県安芸郡江田島町 江田島
中学
- 山脇哲臣 (高知市八軒町 30)
- 湯川啓夫 下関市長府町江下 山口大農
(同市同町松小田一町)
- 横内靖 香川県綾南町 県立主基高校
(香川県仲多度郡琴平町263)
- 横川広美 鳥取市立川町5丁目 鳥取大学
芸生物
- 吉村庸 高知市朝倉乙 775 高知学芸高
校 (高知市朝倉曙町乙 443)
- 米山穰 広島市皆実町 広島大皆実分校
- 渡辺義生 因島市土生町 土生高校
(広島大理 広島市東千田町, 岡山大理 岡
山市津島, 愛媛大文理 松山市持田町)

8. 九州支部

- 秋武和俊 九大理生物
- 秋山文司 熊本市水前寺本町 100 熊本県
蚕業試験場
- 尼川大録 福岡市西新町大西町 4-786 福
岡県立修猷館高校
- 荒木徳蔵 宮崎大学芸附属中学 (宮崎市
神宮町 140-4)
- 石川重夫 熊本大理生物 (熊本市黒髪町
下立田 383)
- 石松英二 福岡県遠賀郡芦屋町 芦屋中学
- 稻田朝次 九大理生物
- 井上覚 熊本大理生物 (同大職員寮)
- 茨木和典 福岡県筑後市和泉西 農林省九
州農業試験場 (福岡県筑後市
久富 県営住宅第8号)
- 今江正知 熊本大理生物
- 岩月善之助 服部植物研究所 (日南市油津
町西町 502)
- 上田英次 大分県宇佐郡院内村大福 東院
内小学校 (同県同郡駅川町樋
田)
- 宇佐美和夫 福岡市 福岡女子大 (福岡市上
辻堂町 11)
- 梅埜国夫 福岡市瀧洲町 福岡高校 (同
市香椎町堂園 635-11)
- 浦山隆司 (宮崎市神宮東町 202-3 柳田
滝夫方)
- 遠藤正喜 大分市碩田中学校 (大分市北新
町6組)
- 大内準 福岡県田川郡香春町 県立田川
高校 (同県同郡同町中津原
田川高校住宅内)
- ◎大木麒一 (佐賀県藤津郡久間村志田原)
- 大城肇 九大農水産植物

大野 照 好	鹿児島大教育生物	竹下 敬 司	福岡県八女郡黒木町 福岡県林業試験場
大橋 裕	九大薬学生薬	田川 日出 夫	九大理生物
岡田 喜 一	長崎大水産 (佐世保市須佐町アパート A-11)	田 中 剛	鹿児島大水産 (鹿児島市田上町 1696-10)
小川 保 喜	福岡学芸大 (福岡県筑紫郡那珂町池田)	谷 口 勇	鹿児島県始良郡加治木町 始良教育事務所
奥 達 夫	鹿児島大文理生物 (鹿児島市上荒田町 1956 赤塚健蔵方)	田村 博 美	鹿児島県出水市武本 県立出水高校
奥 富 清	福岡県田川市伊田 福岡学芸大田川分校	千葉 保 胤	九大理生物
小野 孝	(大分県日田市有田 45)	戸田 良 吉	(熊本市黒髪町下立田 547)
小野 林	福岡市大坪町 九大教養生物 (同市大坪町)	飛田 博 温	宮崎県日南市 市立飫肥中学 (同市飫肥町十文字 斎藤方)
小村 精	九大理生物	*外山 三 郎	長崎大学芸生物 (長崎県長崎市家野原町 466)
片山 義 勇	宮崎大農	中村 和 郎	九大理生物 (八幡市枝光新町 5)
金子 賢 一 郎	福岡学芸大 久留米分校生物	中谷 六 郎	(福岡県京都郡久保村松田)
鴨川 誠	九大理生物	中山 至 大	宮崎大学芸生物 (宮崎局区内大工町 181-3)
茅野 博	九大理生物 (福岡市諸岡公団住宅 232 号)	西 克 久	九大農植 (福岡市多々良町大字名島渡場 1990 吉本方)
楠元 司	鹿児島大教育生物 (鹿児島市薬師町 280)	錦井 徳 之	(熊本市横手町 1011)
久保 淳	(福岡県糸島郡志摩村松隈 767)	西原 幸 男	九大理生物 (福岡市小林町 市営アパート第 5 号)
○瀬瀬 理 一 郎	(福岡県粕屋郡篠栗町山王字平原)	西山 俊 治	福岡県大牟田市草木 三池高校 (大牟田市真導寺町 20)
河野 明 綱	宮崎大農 (宮崎市大島町原ノ島 1608)	二宮 淳 一 郎	別府女子大生物 (別府市北石垣門通寺 82)
小島 均	九大農植 (福岡市馬出浜松町 1-2)	野 口 彰	熊本大理生物
小長光 与 壮	福岡市大坪町 九大教養生物	野沢 治 治	鹿児島大水産 (鹿児島市下荒田町)
小谷 信 夫	九大理生物	野沢 ユ リ 子	鹿児島純心女子学園 (鹿児島市下荒田町 2223)
小林 嘉 光	熊本県天草郡五和町 内野中学 (熊本県本渡市本渡町古川)	野田 健 児	福岡県筑後市大字和泉 農林省九州農業試験場 (同市大字和泉農林省九州農事試験場官舎)
坂口 信 治	鹿児島大文理生物 (鹿児島市川内市川内駅長宿舎)	野見山 光 義	福岡県立稲築高校 (福岡県嘉穂郡碓井町下旧井)
佐藤 仁 蔵	大分県日田郡栄村 五馬中学	芳 賀 恣	九大理生物 (福岡市医学部北町 RA-11)
沢田 武 男	福岡県宗像郡津屋崎町 九大水産実験所	畑 中 健 一	小倉市北方 北九州大生物 (小倉市中曾根 1-687)
三宮 正 信	大分市王子町 大分大学芸生物	初島 住 彦	鹿児島大農
椎葉 昭 二	熊本県水俣市三本松 水俣第二中学	*服部 新 佐	服部植物研究所 (宮崎県日南市おび本町 3-888)
品川 鉄 摩	(長崎県壱岐郡郷之浦町組屋町)	花田 主 計	福岡市荒戸町 福岡学芸大福岡分校生物 (福岡市箱崎昭和町 3205)
清水 正 元	九大農	東 四 郎	鹿児島大文理生物 (鹿児島市呉服町 54)
新 敏 夫	鹿児島大文理生物	東 清 一	熊本大理生物 (熊本市黒髪町坪井 556)
末宗 正 明	大分県中津市如水区是則 東中津中学		
菅原 淨	九大理生物		
鈴木 時 夫	大分市南王子町 大分大学芸		
関岡 行	九大農植 (福岡市大字名島芳町)		
高城 正 勝	鹿児島県熊毛郡中種子町野間 国立衛生試験所薬用植物園種子島分場		

平 川 秋 次 熊本県玉名市 中玉名高校 (同
県玉名郡横島村)

平 田 正 一 宮崎大農

平 松 信 夫 長崎県平戸市田代町 津吉高校

広 瀬 恒 久 熊本県玉名郡豊水村 旭理農業
研究所

藤 木 素 士 熊本市大江町久品寺 191 尚綱
高校 (同市京町本丁 69 佐伯
方)

藤 野 正 義 (長崎県諫早市福田町 2940)

藤 原 勲 佐賀大文理生物

細 川 隆 英 九大理生物 (福岡市鳥飼町 5-
458)

星 乃 洋 二 熊本県本渡市本渡町 本渡中学

本 田 康 人 大分県宇佐郡宇佐町南宇佐 県
立宇佐高校 (同県同郡同町南
宇佐)

前 原 勘 次 郎 (熊本県人吉市寺町 19)

前 田 敏 九大理植

牧 川 鷹 之 祐 (福岡市弥生町 2-35)

松 井 敏 夫 下関市吉見町 農林省水産講習
所

松 下 亀 久 九大理生物

松 島 幹 夫 熊本市神町 400 農林省蚕糸試
験場九州支場

右 田 清 治 長崎大水産

水 谷 正 美 服部植物研究所 (宮崎県日南
市おび本町)

宮 田 逸 夫 九大理生物

村 山 宅 美 佐賀大教育

室 屋 法 曉 九大農造林 (福岡県筑紫郡大
野町大利 325)

森 通 保 熊本県宇土郡宇土町 宇土高校
(同郡不知火村高良 2642)

安 本 徹 熊本市清水町室園 九州女学院

八 尋 正 樹 鹿児島大農

八 戸 正 夫 熊本大教育 (熊本市黒髪町坪
井 51)

山 内 竜 雄 福岡市 三筑北中学 (福岡県筑
紫郡太宰府町 2361)

山 城 守 也 鹿児島県熊毛郡西之表町 種子
島高校

山 下 淳 福岡県筑後市大字和泉 農林省
九州農業試験場大麦育種研究室

山 下 知 治 九大教養生物 (福岡市浦谷十
軒家 50)

山 根 銀 五 郎 鹿児島大文理生物 (鹿児島市
下芝田町 440)

吉 井 甫 九大農

吉 岡 俊 三 福岡市 福岡女子大生物

吉 田 忠 生 九大農水産植物 (戸畑市猪之
坂通 2)

渡 辺 静 馬 熊本県八代郡鏡中学 (八代郡
千丁村古閑出 634)

渡 辺 綱 男 熊本県宇土市段原 320 県立宇
土高校

九大理 福岡市箱崎町, 九大農 福岡市箱
崎町, 熊本大理 熊本市黒髪町, 宮崎大学
芸 宮崎市花殿町, 宮崎大農 宮崎市船塚
町, 長崎大水産 佐世保市崎辺町, 鹿児島
大文理 鹿児島市山下町, 鹿児島大教育
鹿児島市伊敷町, 鹿児島大農 鹿児島市上
荒田町

外 国

△Blaringhem, L. 77, Rue de Saint Pères, Paris
VI^e, France

朴 茂 栄 韓国京城市 京城大学校師範大
学

◎Bonner, J. Division of Biology, Califor-
nia Institute of Technology,
Pasadena 4, Calif., U. S. A.

◎Bünning, E. Botanisches Institut,
Tübingen, Germany

◎Chaney, R. W. Department of Palaeontology,
University of California,
Berkeley 4, Calif., U. S. A.

Chowdhury, N. P. University of Hutment,
No. 19, Near Patel Chest In-
stitute, Central Avenue Road,
Delhi, India

張 鳳 烈 (韓国釜山市西区富民洞一街36)

Durairatnam, M. Department of Fisheries,
Galle Face, P.O. Box 531,
Colombo 3, Ceylon

△Fosberg, F. R. (212 Holmes Run Rd., Falls
Church, Va., U. S. A.)

◎Gates, R. R. (46 Lincoln House, Basil St.
Knightsbridge, London
S.W. 3, England)

呉 秀 栄 韓国大邱師範大学生物学教室

*Graetrex, F. C. c/o Barclays Bank, Salisbury,
South Rhodesia, Africa

◎Heim, R. Muséum National d'Histoire
Naturelle, Rue de Buffon,
Paris V^e, France

姜 濱 求 韓国京城市中区槇忠洞一街 38-
31 高麗大学

姜 悌 源 韓国釜山市 釜山水産大学増殖
学教室

香 村 真 徳 冲縄那覇市 琉球大文理生物

千 景 讓 中国台北市 台湾大学理学院植
物学系 (台北市温州街 18 巷
16 号)

Kang, J. D. 韓国釜山市東萊区 中央園芸研
究所 (釜山市中央郵政局私書函
第 81 号)

- Kaul, K. N. The Director, National Botanic Gardens, Lucknow, India
- 金 倬 熙 韩国京城市筆洞 東国大学文理科大学生物学科
- 金 遵 敏 韩国京城市 京城大学校師範大学
- 金 鼎 錫 韩国水原市 林業試驗水原育種支場
- 金 完 圭 韩国釜山東萊区 中央園芸技術院耕種第一場
- 河 永 亡 韩国京城市城北区安岩洞高麗大学校生物科
- 洪 淳 佑 韩国京城市城北区安岩洞高麗大学校生物科
- 洪 元 植 韩国京城市 Catholic 大学医学部予科生物科
- 權 炳 金 韩国大邱市 慶北大学師範大学生物学教室
- Kondo, K. C. 2457 Liliha St., Honolulu 17, Hawaii, U. S. A.
- △Lindquist, B. Batanic Gardens, Göteborg, Sweden
- ◎Lundegårdh, H. Växsfysiologisk Institutionen, Högskolan, Uppsala 7, Sweden
- △Motte, J. Institute Botanique, Montpellier, Hérault, France
- 任 綱 彬 韩国水原市 京城大学校農科大学生物学科
- 中 村 直 冲繩那霸市 琉球大学生物学教室
- ◎羅 宗 洛 中華人民共和国上海岳陽路 中国科学院植物生理研究所
- 李 溶 基 韩国釜山市東萊区 釜山大学校文理科大学
- 李 龍 雨 韩国京城市東大門区清凉里 林業試驗場
- 李 德 鳳 韩国京城市城北区安岩洞高麗大学校生物科
- 李 範 勲 韩国釜山市 釜山大学校文理科大学生物学科
- 李 敏 載 韩国京城市 京城大学校文理科大学生物学科
- 李 潤 浩 韩国春川市 中央路江原道教育研究所
- 崔 斗 文 韩国忠清南道忠南公州師範大学生物
- 崔 俊 浩 韩国釜山市 釜山大学
- Sampath, S. Cytologist Central Rice Research Institute, Cuttack 4, Orissa, India
- 佐 久 本 敏 冲繩那霸市 琉球大学文理学部生物学教室
- 車 東 爽 韩国京城市城北区安岩洞高麗大学校生物科
- 島 袋 守 成 冲繩那霸市 琉球大学生物学教室
- 朱 尙 寧 韩国釜山市東大新洞 1-60
- 襄 淵 韩国大邱市芳村洞 850 慶北農事院
- 謝 万 權 台湾省立農學院森林植物研究室
- 新 城 和 治 冲繩那霸市 琉球大学文理学部生物学教室
- ◎Skottsberg, C. Riksherbariet, Naturhistoriska Riksmuseet, Stockholm 50, Sweden
- ◎St. John, H. Department of Botany, University of Hawaii, Honolulu 14, Hawaii, U. S. A
- 玉 木 拓 冲繩那霸市三原区 7 班 49
- 鄭 佐 秀 韩国慶南晋州市晋州農科大学圖書館
- △郑 万 鈞 中華人民共和国 南京林学院
- 鄭 玟 培 韩国春川市 春川農科大学
- 鄭 英 昊 韩国京城市 京城大学文理科大学植物学科
- 丁 年 基 韩国釜山市東萊区 金井国民学校
- 鄭 東 民 韩国釜山市温泉洞 中央園芸試驗場
- 鄭 東 敏 韩国釜山市温泉洞 中央園芸試驗場
- 鄭 寅 初 韩国釜山市 釜山大学藥学大学
- ◎Thimann, K. V. The Botanical Laboratories, Harvard University, Cambridge, Mass., U. S. A.
- 友 利 哲 夫 冲繩那霸市 琉球大学文理学部生物学教室
- △Walker, E. H. 7413 Holly Avenue, Takoma Park 12, Maryland, U.S.A.
- △Ullrich, H. Institut für Landwirtschaftliche Botanik, Bonn, Germany
- Jia-Hsi Wu Botany Department, Washington University, St. Louis, Mo., U. S. A.
- 屋 良 和 子 冲繩那霸市首里 琉球大文理

Relation between the Distribution of Aquatic Hyphomycetes in Japanese Lakes and Lake Types

by Shizuo SUZUKI* and Hiroyoshi NIMURA**

Received May 14, 1960

Many excellent works on aquatic Hyphomycetes of fresh-water lakes have been published in Europe and America during the past two decades. Among the contributions the writers recall the names of Ingold¹⁻¹¹), Ranzoni^{12, 13}), Nilsson¹⁴), Tubaki¹⁵) and others^{16, 17}). These investigators, however, studied mainly the distribution and taxonomy of the fungi, and paid little attention to the ecology of them. The writers have engaged in floristic and ecological studies of the aquatic fungi for several years, emphasizing the relation of the distribution of aquatic Hyphomycetes and the physico-chemical properties of lake water.

Experimental method

The aquatic Hyphomycetes inhabits decaying leaves of trees fallen in lakes. Samples of these leaves were taken from lakes and those in stoppered bottles brought back into the laboratory. Six to ten leaves were collected from each lake. The collected materials were placed in petri dishes partially filled with autoclaved tap water. The specimens were allowed to stand at room temperature (15-25°) for 24 hours or more, then the conidia were found abundantly on the submerged leaves. The identification of species was carried out using a low power microscope. To make a detailed observation the fungus was stained with lactic phenol cotton blue.

Distribution of aquatic Hyphomycetes

The aquatic Hyphomycetes were widely distributed in the Japanese lakes. The distribution of the fungi, however, differed considerably with lake types (Table 1). On the basis of the qualitative data obtained, it is evident that the fungus flora was very rich in the harmonic lakes, while it was very poor in the dystrophic and acidotrophic lakes. In latter case, however, certain peculiar species adapting to the disharmonic water were found in large quantity.

Although eleven species were isolated from the harmonic lakes, *Tetrachaetum elegans*, *Articulospora tetracladia*, *Lemonniera aquatica* and *Anguillospora longissima* were the most prevalent in the lakes of this type. No aquatic Hyphomycetes, however, could be found in Lake Chūzenjiko and Towadako, which belong to the oligotrophic type.

On the other hand, aquatic Hyphomycetes were very slight or non-existent in the dystrophic lakes. As the lake water of this type contained large amounts of humic substances and reacted strongly acid, the physico-chemical specificities of the water may perhaps depress the activity of the fungi. *Varicosporium elodeae* seems to adapt to the dystrophic lakes. This species was found in neither the harmonic nor the acidotrophic lakes.

It is a very interesting fact that *Tricladium gracile* var. *oxyphilum* and *Anguillo-*

* Department of Microbial Chemistry, Faculty of Pharmacy, Tokyo College of Science.

** Botanical Institute, Faculty of Science, Tokyo University of Education.

Table 1. The distribution of aquatic Hyphomycetes and lake types of Japanese lakes.

Lake types	Lakes	Localities*	<i>Anguillospora longissima</i>	<i>Articulospora tetracladia</i>	<i>Clavariopsis aquatica</i>	<i>Flagellospora curvula</i>	<i>Lemonniera aquatica</i>	<i>Lunulospora curvula</i>	<i>Margaritispora aquatica</i>	<i>Tetrachaetium elegans</i>	<i>Tetracadium marchalianum</i>	<i>Tricladium gracile</i>	<i>T. gracile</i> var. <i>oxyphilum</i>	<i>Tricladium splendens</i>	<i>Triscelophorus monosporus</i>	<i>Varicosporium elodeae</i>
Harmonic	<i>Tsukinuma</i>	A	○													
	<i>Kagaminuma</i>	A	○													
	<i>Shigenuma</i>	A	○	○												
	<i>Naganuma</i>	A	○	○												
	<i>Hyōtannuma</i>	A	○	○												
	<i>Yanaginuma</i>	A	○	○												
	<i>Nishiyanaginuma</i>	C	○	○												
	<i>Jimushonuma</i>	C	○	○												
	<i>Yarokunuma</i>	C	○	○												
	<i>Otomenuma</i>	C	○	○												
	<i>Kidoike</i>	F	○	○												
	<i>Tsutanuma</i>	F	○	○												
	<i>Biwaïke</i>	F	○	○												
	<i>Maruïke</i>	F	○	○												
Dystrophic	<i>Harunako</i>	D		○												
	<i>Soharako</i>	D		○												
	<i>Yamanakako</i>	G		○												
	<i>Chūzenjiko</i>	E		○												
	<i>Towadako</i>	A														
	<i>Suirennuma</i>	A														
	<i>Ichinuma</i>	F														
	<i>Nagaike</i>	F														
	<i>Misumaïke</i>	F	○													
	<i>Shijūhachiike</i>	F														
	<i>Shibuike</i>	F														
	<i>Ozegahara pond</i>	D														
	<i>Ayamedaira moor</i>	D														
	<i>Kamagaike</i>	F														
<i>Kurumayama moor</i>	F	○														
Acidotrophic	<i>Katanuma</i>	B														
	<i>Akadoronuma</i>	C														
	<i>Akanuma</i>	C														
	<i>Hyōtannuma</i>	C														
	<i>Kokenuma</i>	C														
	<i>Ōnumaïke</i>	F	○													
	<i>Aodoronuma</i>	C														
	<i>Rurinuma</i>	C														
	<i>Aonuma</i>	C	○													
	<i>Akanuma (Hakkōda)</i>	A														
	<i>Bentennuma</i>	C														
	<i>Bishamonnuma</i>	C	○													
	<i>Midoronuma</i>	C	○													

* Prefectures: A Akita, B Iwate, C Miyagi, D Fukushima, E Ibaraki, F Tochigi, G Gunma, H Saitama, I Chiba, J Tokyo, K Kanagawa, L Niigata, M Toyama, N Ishikawa, O Fukui, P Gifu, Q Shizuoka, R Aichi, S Mie, T Shiga, U Kyoto, V Osaka, W Hyogo, X Nara, Y Wakayama, Z Tottori, AA Shimane, AB Izumi, AC Yamaguchi, AD Hiroshima, AE Okayama, AF Kagawa, AG Tokushima, AH Kochi, AI Fukuoka, AJ Saga, AK Nagasaki, AL Kumamoto, AM Oita, AN Kagoshima, AO Okinawa.

* Prefectures: A, Aomori; B, Miyagi; C, Fukushima; D, Gunma; E, Tochigi; F, Nagano; G, Yamanashi.

spora longissima dominated in the acidotrophic lakes. The distribution of the former species was restricted in the acidotrophic lake water containing large amounts of mineral elements¹⁸). No aquatic fungus was found in Lake Katanuma and Akadoronuma. This is caused by the strong acidity as well as by the chemical specificity of lake water¹⁸).

Among many factors determining the distribution of species in lakes, the physico-chemical properties of the lake water are considered to be the most essential. The water of the dystrophic lakes revealed in tests strong acidity and contained large amounts of humic substances, while the water of the acidotrophic lakes are very rich in inorganic acids. The strong acidity of the water of both lake types may be too toxic for the aquatic fungi to distribute in them. However, the mineral salts such as sulfate of iron and calcium, were very effective on the growth of the aquatic fungi. According to the water analysis of the acidotrophic lakes, some of mineral elements were measured as follows: SO_4 , 75-442; Fe, 0.01-278; Mn, 1.0-9.1; Ca, 32-495; Cl, 5-202 in mg./l. These amounts of mineral elements may provide the factors that decide the distribution of aquatic Hyphomycetes.

Physiological specificity of the fungi and lake type

On the distribution of aquatic Hyphomycetes in many Japanese lakes, it is clear that the distribution is determined by the acidity, mineral components and some other factors of the lake water. To make clear the specificity of lake water to the aquatic fungi, some laboratory experiments were carried out.

The aquatic Hyphomycetes were inoculated on yeast extract glucose agar. Under such conditions the mycelial growth occurred but never the conidia formation. A piece of mycelium of the fungi was then submerged in the lake water of different lake types. After this was allowed to stand for a day or two at room temperature, the formation of conidia was observed (Table 2).

The conidia formation differed with the water of different lake types and different species. The conidia were quickly formed in the water of the harmonic lakes, while they were very slight or non-existent in the dystrophic and acidotrophic lake water.

The conidia of *Tricladium gracile*, *Lemonniera aquatica* and *Anguillospora longissima* were formed even in the water of the acidotrophic lakes. These species were frequently found in the inorganic acidotrophic lakes in Japan. No conidia formation, however, was observed in strongly acid water of Lake Katanuma and Akadoronuma. On the other hand, the conidia of *Clavariopsis aquatica*, *Tetrachaetum elegans* and *Articulospora tetracladia*, which were found only in the harmonic lakes, were not formed in the water of the acidotrophic lakes. The result was in accord with the observations made by the writers in the natural lakes.

The results obtained in dystrophic lakes are very complicated. The conidia formation of all species occurred in the water of Lake Nagaike, while the water of ponds of Kinunuma High Moor very toxic to some fungi. The disharmony in the distribution of fungi may be caused by the humic substances dissolved in lake water.

Table 2. The conidia formation of aquatic Hyphomycetes in the lake waters of diverse lake types.

Lake types	Lakes	<i>Anguillospora longissima</i>	<i>Articulospora tetractadia</i>	<i>Clavariopsis aquatica</i>	<i>Lemonniera aquatica</i>	<i>Tetrachaelum elegans</i>	<i>Tricladium gracile</i>
Harmonic	Ōsawanuma	##	##	##	##	##	##
	Yanaginuma	##	##	##	##	##	##
	Nakanuma	##	##	##	##	##	##
	Harunako	##	##	##	##	##	##
	Yamanakako	##	##	##	##	##	##
Dystrophic	Nagaike	##	##	##	##	##	##
	Shibuike	+	##	-	+	##	+
	Shijūhachiike	##	##	-	##	##	##
	Ozegahara pond	+	##	+	##	-	+
	Kinunuma	-	##	-	##	##	+
Acidotrophic	Katanuma	-	-	-	-	-	-
	Akadoronuma	-	-	-	-	-	-
	Akanuma	##	-	-	+	-	+
	Rurinuma	##	-	-	##	-	##
	Aonuma	##	+	+	##	+	##
	Bishamonnuma	##	+	##	##	-	##

Summary

The relation between the distribution of aquatic Hyphomycetes and the lake types was studied in some Japanese lakes. The aquatic Hyphomycetes were very rich in both quantity and number of species in the harmonic lakes, while they were slight or non-existent in the dystrophic and acidotrophic lakes. *Tricladium gracile* var. *oxyphilum* and *Anguillospora longissima* were the dominating species in the acidotrophic lakes, and *Varicosporium elodeae* was found only in the dystrophic lakes.

The experiments were carried out in the laboratory on the effect of the lake water upon the conidia formation of the fungi. The conidia were easily produced in the water of the harmonic lakes, but never in some acidotrophic one. *Anguillospora longissima*, *Lemonniera aquatica* and *Tricladium gracile* seem to be suitable to the acidotrophic water containing large amounts of mineral elements. The results were in accord with the observation in the natural lakes.

The writers wish to express their thanks to Prof. H. Indoh and Prof. H. Ito for their instructive guidance and advice. Also to Dr. S. Ichimura, Prof. T. Tatsuno

and T. Matsumoto, the writers are indebted for much valuable advice during this work.

References

- 1) Ingold, C. T., Trans. Brit. Mycol. Soc. **25**: 339 (1942). 2) —, *ibid.* **26**: 104 (1943). 3) —, *ibid.* **26**: 148 (1943). 4) —, New Phyt. **40**: 139 (1943). 5) —, Trans. Brit. Mycol. Soc. **27**: 35 (1944). 6) —, Proc. Linn. Soc. Lond. **157**: 43 (1945). 7) —, Trans. Brit. Mycol. Soc. **32**: 341 (1949). 8) —, *ibid.* **35**: 158 (1952). 9) —, *ibid.* **35**: 66 (1952). 10) —, Rep. Internat. Bot. Congress, Sect. **19**: 62 (1954). 11) —, and Cox, V. J., Trans. Brit. Mycol. Soc. **40**: 155 (1957). 12) Ranzoni, F. V., Falowia **4**: 353 (1953). 13) —, Amer. Journ. Bot. **43**: 13 (1956). 14) Nilsson, S., Sven. Bot. Tidskr. **52**: 291 (1958). 15) Tubaki, K., Bull. Nat. Sci. Mus. Tokyo **3**: 249 (1957). 16) Waid, J. S., Trans. Brit. Mycol. Soc. **37**: 420 (1954). 17) Willen, T., Bot. Not., 111, Fasc. **2**: 431 (1958). 18) Suzuki, S., and H. Nimura, Bot. Mag. Tokyo **73**: 360 (1960).

摘 要

鈴木静夫・二村坦孝：水棲不完全菌類の分布と湖沼型

日本の 42 個の湖沼の水棲不完全菌類を調査し、特に湖沼型と種類の分布との関係を明らかにした。水棲不完全菌類は調和型の湖沼には種類が豊富であるが、酸栄養湖や腐植栄養湖では種類が少ない。すなわち、酸栄養湖には特異な水に適応した *Tricladium gracile* var. *oxyphilum* と *Anguillospora longissima* が優占し、腐植栄養湖には *Varicosporium elodeae* がもっとも普通に見られるが、調和湖に見られる種類はほとんど棲息しない。

異なった湖沼型に属する湖沼の水に純粋に培養した水棲不完全菌類の菌糸を入れ、分生子の形成の有無を観察した。その結果、調和湖の湖水中では各種類ともよく分生子が形成されたが、酸栄養湖の湖水中ではこの型の湖沼に多く見られる *Tricladium gracile*, *Anguillospora longissima*, *Lemonniera aquatica* の 3 種だけが分生子を生じ、これらの種類が多量の無機塩類を含有している酸栄養湖の水に適応していることが明らかになり、野外での観察結果が裏づけられた。しかし、腐植栄養湖の湖水中では比較的良好に分生子が形成され、実際に湖沼にはほとんど菌類が棲息しておらず、両者の結果が一致しない。（東京理科大学薬学部微生物化学教室；東京教育大学理学部植物学教室）

Stimulative Effect of Certain Specific Bacteria upon Mycelial Growth and Fruit Body Formation of *Agaricus bisporus* (Lange) Sing.

by Takashi URAYAMA*

Received May 14, 1960

Some fungi may influence the reproduction of another. Porter¹⁾ reported stimulating effect of some fungi upon reproduction of *Alternaria* and *Helminthosporium*. Benedek²⁾ and Hanzen³⁾ demonstrated that *Bacillus weidmaniensis* greatly stimulated macroconidia production in *Microsporium audouini*. Reproduction of *Sordaria fimicola* was stimulated by placing *Aspergillus regulosus* to the same medium (Barnett and Lilly⁴⁾). However, the author could find no valuable bibliography on the mutual effect between microorganisms and hymenomycetes.

It was shown in a previous paper⁵⁾ that the presence of *Bacillus Psilocybe* 1 (a species of *Bacillus* inducing the fruiting in *Psilocybe panaeoliformis* Murrill and some hymenomycetes, cf. Urayama^{5,6)}) increased density of mycelial colony in *Agaricus bisporus* (Lange) Sing. on yeast-sucrose agar medium in a test tube but did not induce the fruiting.

It is the purpose of the present experiment to investigate influence of the bacteria upon fruit body formation of *Agaricus bisporus* using various cultural media and vessels.

Material and Method

White species of *Agaricus bisporus* (Lange) Sing. was used as the material. Horse manure compost corrupted sufficiently was adjusted to about pH 6.7 by scattering calcium biphosphate powder. At first, two kilograms of the compost were put in a 10 liter Erlenmeyer flask (ca. 30.5 cm. in diameter of the base) and then the surface was flattened by light pressing down to keep the height 6~7 cm. After a while, a spawn (ca. 3.5 cm³. in volume) was buried under the middle surface of the compost. Several days after initiation of the mycelial growth, the compost was covered with small grain of the sand with some loam (the layer of 2—2.5 cm. in height). These flasks with cotton stopper were placed in a dark room with 70~82% relative humidity at 10~21°.

Only bacteria cultured on peptone-sucrose agar medium** or yeast-sucrose agar one*** were mixed with sterilized water. The suspension was sprayed in and on the compost once before setting it in the flask and once more before putting the soil on it (20 days after the setting).

Secondly, a glass pot (12 cm. in depth, 20 cm. in diameter of the base) and a wooden box**** (35 cm. deep×25 cm.×50 cm.) were prepared by the same treatment as in Erlenmeyer flask and these were placed in the dark room with 70~80% relative humidity at 15~20°.

* Department of Biology, Miyazaki University, Miyazaki, Japan.

** Four g. peptone, 20g. sucrose, 0.25 g. KH₂PO₄, 0.25 g. CaCl₂·6H₂O, 0.25 g. MgSO₄·7H₂O, 0.12g. KCl, trace FeCl₃, 20 g. agar, 1000 cc. distilled water.

*** Five g. dried yeast ("Ebios"), 20 g. sucrose, 20 g. agar, 1000cc. distilled water.

**** In this case, layer of the compost was 12-13 cm. thick.

Results and Discussion

In the experiments using the flask, the mycelial density was promoted by the bacterial spraying (Photo. 1), however the fruiting was not induced as this was also the case in test tube experiments⁵).

Inadequate aeration due to cotton stopper was avoided by the experiments with glass pot or wooden box. The mycelial density in the glass pot (Photo. 2) was increased, and the fruit body formation was only slightly promoted by the spraying,

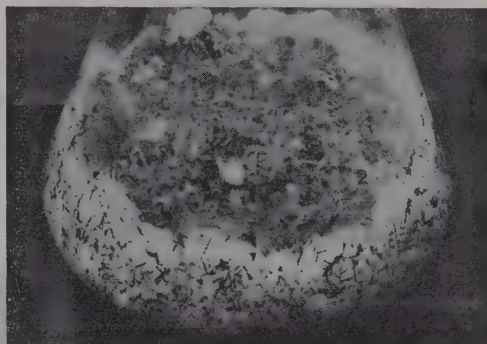
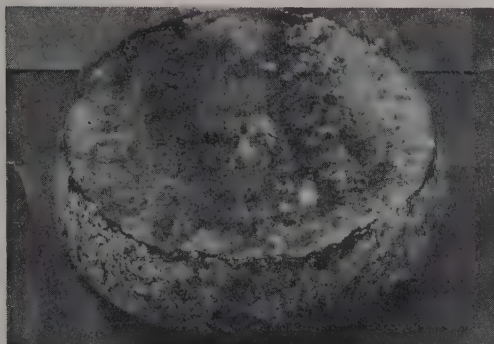


Photo. 1. Stimulative effect of the bacteria upon the mycelial density on the horse manure compost in a Erlenmeyer flask. Observation, 37 days after the mycelial inoculation. Left, a control flask without spraying the bacterial suspension.

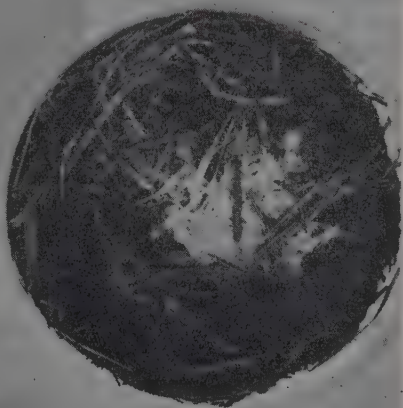


Photo. 2. Stimulative effect of the bacteria upon the mycelial growth on the horse manure compost in a glass pot. Observation, 23 days after the mycelial inoculation. Left, a control pot without spraying the bacterial suspension.

that is, the bacterial effect upon the fruiting was not conspicuous as the fruit bodies formed on the bed were relatively few in this case (Photo. 3). When these mycelia with the compost were replaced in a wooden box (see below), the fruiting again occurred.

It is probable that the mycelia in the compost which is enclosed with glass wall and soil or cotton stopper does not produce abundant fruit bodies.

On the other hand, the mycelia cultured in a wooden box produced many fruit

bodies, especially the mycelia sprayed with the bacterial suspension initiated the fruiting 5~10 days earlier than ones without spraying (Photo. 4). Total production of the fruit bodies* was also increased about 30% by the treatment.



Photo. 3. Fruit body formation on the cultural bed sprayed with the bacterial suspension in a glass pot.

Photo. 4. Stimulative effect of the bacteria upon the fruit body formation on the bed of horse manure compost covered with the soil in a wooden box. Observation, 43 days after the mycelial inoculation. Left, a control box without spraying the bacterial suspension.



The mycelial density increased by the spraying is likely to be due to an increase of available constituents in the compost. Though the mycelial density is not always accompanied with the fruiting, it seems that the fruiting may be connected with any process of the mycelial metabolism leading to the increased mycelial growth.

Summary

By spraying *Bacillus Psilocybe* 1, mycelial density and total production of the fruit bodies in *Agaricus bisporus* (Lange) Sing. were increased and initiation of the fruiting became earlier.

The author wishes to express his appreciation to Prof. S. Toyama of Miyazaki University for his kind encouragement throughout this investigation. Thanks are also due to Mr. I. Tani, for his kind aid.

* Having 2-3 cm. diameter of the hut.

References

- 1) Porter, C. L., Amer. J. Bot. **11**: 168 (1924). 2) Benedek, T., Mycol. **35**: 222 (1943).
3) Hanzen, E. L., ibid. **39**: 200 (1947). 4) Barnett, H. L., and Lilly, V. G., Amer. J. Bot. **34**: 196 (1947).
5) Urayama, T., Mem. Fac. Liber. Arts. Educ., Miyazaki Univ. **9**: 393 (1960). 6) —, Bot. Mag. Tokyo **70**: 29 (1957).

摘 要

浦山隆司： ハラタケ(マツシエルーム)の菌糸生長と子実体形成におよぼす細菌の刺激効果

シビレタケ一種の子実体形成を促進する細菌 *Bacillus Psilocybe* 1 (仮称) の懸濁液を散布することにより、ハラタケの菌糸生長と子実体形成量を増進することができた。また、子実体形成開始も早められた。(宮崎大学学芸学部生物学教室)

Effect of Low Temperature on Bacterial Oxidations*

by Sumiko NAKAO**

Received July 13, 1960

The formation of enzymes in bacterial cells is known to be susceptible to the thermal conditions of culturing. In 1943 E. F. Gale¹⁾ reported that in a strain of *Escherichia coli*, the amino acid decarboxylase formation was more strongly stimulated when the bacterium was cultured at 30° than at 37°, while *Clostridium welchii* showed more enhanced formation of this enzyme at 37° than at 30°.

Recently, S. Sasaki and S. Usami²⁾ reported that in *Proteus vulgaris* which had grown more rapidly at 37° than at 24°, the formation of amino acid oxidase was more enhanced at 24° than at 37°. S. Usami *et al.*³⁾ have reported that in germinated embryos of wheat, the activity of a certain copper enzyme increased gradually on low temperature treatment, while that of cytochrome oxidase decreased. These results suggest that by low temperature treatment, the copper enzyme becomes operative as the terminal oxidase in place of cytochrome oxidase.

In an experiment with *Neurospora crassa*, N. H. Horowitz and his collaborator⁴⁾ reported that cells showed practically no tyrosinase activity when cultured at 35° on a medium which was highly favourable to the production of its activity at 25°.

The optimum temperature for the growth of bacteria lies usually between 30° and 37°, and therefore the temperature dependence of the oxidative activity and enzyme formation in bacteria has usually been studied around this range of temperature. There are, however, bacteria known to be capable of growing at a temperature as low as 0°. With these microorganisms, the range of temperature to be investigated in this respect becomes much more extended.

It is also known that enzyme activities change with cell age. According to S. Sasaki and S. Usami²⁾, the activity of the amino acid oxidizing system of *Proteus vulgaris* was greater in earlier stages of cell growth. Moreover, there are reports that the capacity for enzymatic adaptation is related to the age of cells^{5, 6)}. The purpose of the present investigation is to analyse the sensitivity to low temperature of the oxidizing activity of a bacterium at various stages of growth.

Methods

Pseudomonas aeruginosa, which was grown on 2 per cent agar slant (pH 7.2) containing 1 per cent peptone-broth, was used.

For the first series of experiments, the bacterium was incubated at 30° for 30 min., and then placed in an ice box at 5–7° for several days. During this low temperature treatment, there was no detectable change in cell number. After the treatment, the bacterium was grown at 30° for 15 hours. The cells were harvested and suspended in 0.9 per cent NaCl solution, washed twice by centrifugation, and resuspended in the saline solution, to be used in the experiments.

For the second series of experiments, the bacterium was grown at 30°, and cells in the exponential (8 hrs.) or the stationary (15 hrs.) phase of growth were placed in

* Part of this study was already presented at the Annual Meeting of Botanical Society of Japan held at the University of Tokyo in October, 1957.

** Department of Pharmacology, Fukushima Medical College, Fukushima, Japan.

the ice box for several days. Cell suspensions were made as described above.

For the third series of experiments, the bacterium was grown at 10°, 24° and 30° and after various times of incubation, bacterial suspensions were similarly prepared.

The amount of the organism in a suspension was given in terms of cellular dry weight. The growth of the organism was measured by viable counts or by turbidity. A Warburg apparatus was used for the respiratory measurements. Oxygen uptake was measured with alkali in the centre well and the air in the gas space. Substrates and inhibitors were added from the side arm. The concentration of substrates was M/30, unless otherwise stated. The measurement of dehydrogenase activity was carried out by the Thunberg method at 30° with methylene blue as acceptor.

Results

(1) Effect of low temperatures on the bacterium in initial phase of growth.

a) Effect of low temperature treatment on respiration.

The effect of low temperature treatment (5-7°) on the respiration of the bacterium in the initial stage of growth is shown in Fig. 1, in which the Q_{O_2} values for the oxidation of various substrates are plotted against time of cold treatment (days).

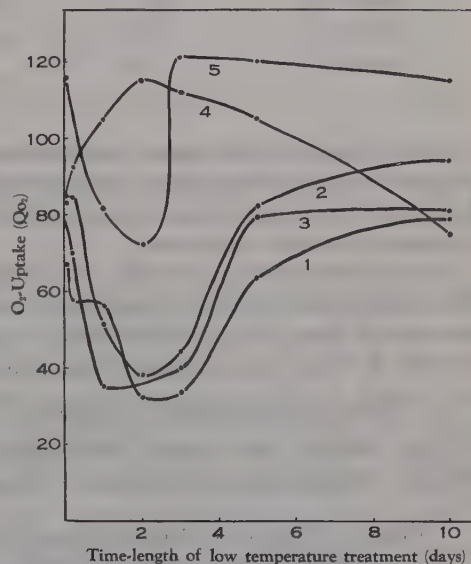


Fig. 1. Effect of low temperature on respiration of *Ps. aeruginosa* in initial phase of growth.

Each vessels contained 0.5 ml. of bacterial suspension, 0.5 ml. of M/4 phosphate buffer (pH 7.2) and 0.4 ml. of substrate in main room; 0.5 ml. of 15 per cent NaOH in centre cup. Total volume was made up to 2.5 ml. with distilled water. Reaction temperature, 30°.

Curve 1, acetate; curve 2, succinate; curve 3, malate; curve 4, formate; curve 5, lactate.

The activities to oxidize acetate, succinate, malate and lactate were found to be at the lowest level (about 40-60 per cent of the control level) when the cells had been exposed to low temperature for a short period (about 2-3 days), to be somewhat recovered on further low temperature treatment. The oxidation of formate, on the other hand, was remarkably enhanced by a short temperature treatment (1 to 5 days long) and showed a maximal value when treated for 2 days which, as described above, correspond to the period for minimum level of oxidation of other acids examined. The oxidative activities toward amino acids (L-glutamate, DL-alanine, DL-phenylalanine, L-leucine and L-histidine) and aromatic substances (pyrogallol, *p*-phenylenediamine and L-tyrosine) remained unaffected by this treatment.

b) Effect of low temperature treatment on dehydrogenase activity.

The next experiments were designed to elucidate the mechanism of the decrease

in activities of oxidation of the organic acids studied above (see Fig. 1). Table 1 shows the effect of low temperature on the dehydrogenase systems.

Table 1. Effect of low temperature on dehydrogenase activity of *Ps. aeruginosa* in initial phase of growth.

Each Thunberg tube contained 1.0 ml. of bacterial suspension, 1.0 ml. of M/4 phosphate buffer (pH 7.2) and 1.0 ml. of methylene blue of adequate concentration corresponding to dehydrogenase activity studied. Final concentration of substrates, M/30; total volume, 5.0 ml.; reaction temperature, 30°; Q_{mb}, micromoles of methylene blue reduced per hour by 1 mg. of dry weight of bacterial cells.

Substrate	Low temperature treatment (days)			
	0	1	2	3
Acetate	0	0.02	0	0.07
Succinate	0.08	0.11	0.14	0.12
Malate	0	0.01	0.07	0.12
Formate	2.04	2.95	4.91	3.48
Lactate	0.12	0.12	0.39	0.28

Q_{mb}

The activity of the organic acid dehydrogenases gradually increased on cold treatment and reached the highest level when the aerobic oxidation of these substances showed the lowest level (cf. Fig. 1). With more prolonged cold treatment, the dehydrogenase activities decreased.

From these findings, namely, the difference in effects of low temperature upon the oxygen uptake and the methylene blue reduction, it may be concluded that low temperature does not inhibit the dehydrogenase but inhibits the terminal oxidase in the cells at their initial phases of growth.

c) Effect of heavy-metal inhibitors.

The effect of heavy-metal inhibitors on the respiration of the bacterium treated with low temperature was studied. The substrate used was succinate. The results

Table 2. Effect of various inhibitors on respiration of cells treated with low temperature in initial phase of growth.

The reaction mixture was the same as that shown in Fig. 1. The experiment of CO inhibition was carried out in gas phase of 90 per cent CO and 10 per cent O₂. Reaction temperature, 30°. Numerals indicate the percentage of inhibition to the control.

Inhibitor	Low temperature treatment (days)			
	0	2	5	12
M/1000 KCN	90	86	79	81
M/250 8-Hydroxyquinoline	79	76	—	48
M/500 Salicylaldehyde	87	76	—	44
90% CO	0	0	0	0

are shown in Table 2. The oxidation of succinate by the cells not treated with low temperature was markedly inhibited by cyanide, 8-hydroxyquinoline and salicylaldehyde but not by 90 per cent carbon monoxide. On low-temperature treatment, the inhibition by cyanide, 8-hydroxyquinoline and salicylaldehyde decreased gradually, but again carbon monoxide did not evoke any inhibition. It has been reported that intact cells of *Ps. aeruginosa* are insensitive toward CO².

d) Effect of pH.

The effect of pH on succinate oxidation by the bacterium was investigated under the conditions similar to those described above. Table 3 shows that the activity of

Table 3. Effect of pH on activity of succinate oxidation of cells treated with low temperature in initial phase of growth.

Experimental condition was the same as that in Fig. 1, excepting pH.

pH	Low temperature treatment (days)			
	0	2	5	12
5.4	89	56	112	131
7.2	96	52	91	105
8.0	96	53	91	—

succinate oxidation by the untreated cells is lower at pH 5.4 than at pH 7.2 and pH 8.0, whereas, on prolonged treatment, the activity becomes higher at pH 5.4 than at pH 7.2 and pH 8.0. No difference could be observed between values for pH 7.2 and pH 8.0.

(2) Effect of low temperature on the bacterium in exponential and stationary phases of growth.

a) Effect of low temperature treatment on respiration.

Enzyme activities and enzymic adaptability of bacteria are known to change with the growth phase^{1,2, 5,6}). This fact leads to the inference that the sensitivity to low temperature may also change during the course of growth. In order to test the inference, the sensitivity to low temperature of the cells in the exponential and the stationary phases of growth was compared with that in the initial phase of growth. Fig. 2 shows the effect of low temperature treatment on the respiration of bacterial cells collected at the exponential phase of growth at 30°. As shown in Fig. 2A, the activity of oxidation of organic acids and amino acids decreased progressively with the lapse of time of treating the bacteria at low temperature (5°). On the contrary, there was little decrease in activity of oxidation of aromatic substrates such as catechol, pyrogallol, L-tyrosine and DL-phenylalanine (Fig. 2B). In some of them, there occurred even a temporary increase in activity. The experiments with the cells in the stationary phase of growth (Fig. 3) gave qualitatively analogous results.

b) Effect of low temperature on succinic dehydrogenase and cytochrome oxidase.

A study was made on the effect of low temperature treatment on succinic dehydrogenase and cytochrome oxidase. Tables 4 and 5 indicate the decrease in both enzyme activities of bacterial cells treated at a low temperature at their exponential and stationary phases of growth.

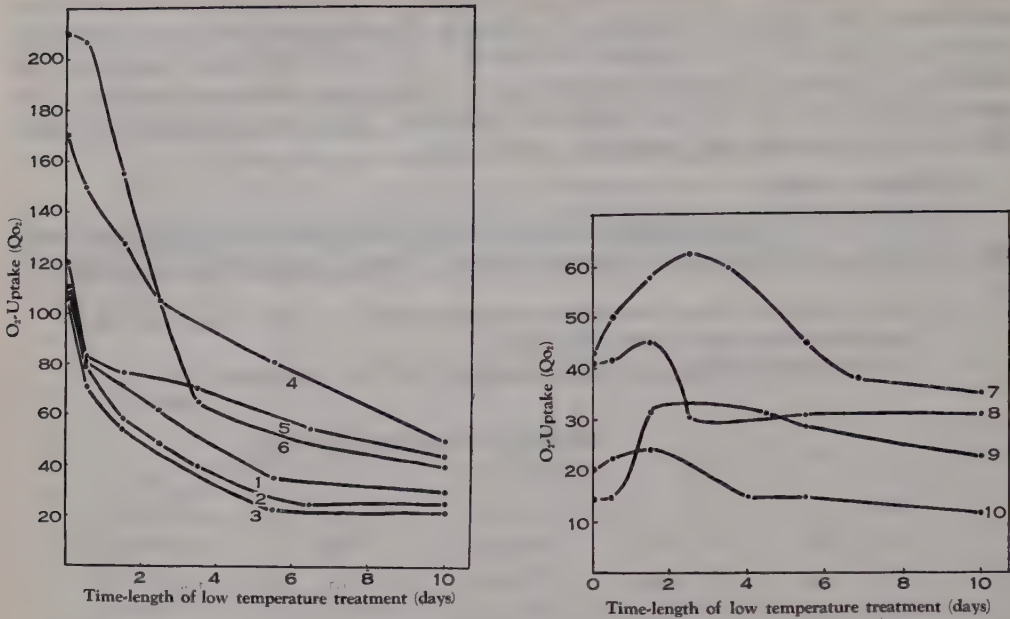


Fig. 2. Effect of low temperature on respiration of *Ps. aeruginosa* in exponential phase of growth.

Experimental condition was the same as that in Fig. 1.

- A. Curve 1, acetate; curve 2, succinate; curve 3, formate; curve 4, lactate; curve 5, L-glutamate; curve 6, DL-alanine.
- B. Curve 7, L-leucine; curve 8, M/250 L-tyrosine; curve 9, M/500 catechol; curve 10, DL-phenylalanine.

Table 4. Effect of low temperature on succinic dehydrogenase activity of *Ps. aeruginosa* in exponential phase of growth.

Experimental condition was the same as that in Table 1.

	Low temperature treatment (days)		
	0	5	10
Succinic dehydrogenase activity (Mb mg./min./mg dry wt. of cells)	0.0039	0.0026	0.0017

Table 5. Effect of low temperature on *p*-phenylenediamine oxidizing activity of *Ps. aeruginosa* in stationary phase of growth.

Experimental condition was the same as that in Fig 1. Final concentration of *p*-phenylenediamine was M/50.

	Low temperature treatment (days)		
	0	5	10
<i>p</i> -Phenylenediamine oxidizing activity (Q _{O2})	34	22	—

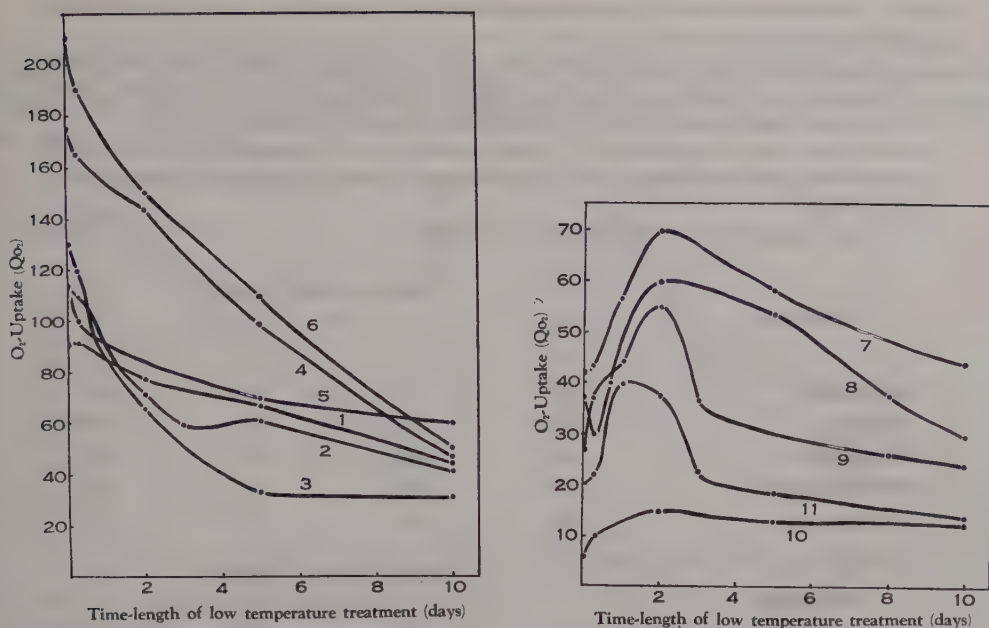


Fig. 3. Effect of low temperature on respiration of *Ps. aeruginosa* in stationary phase of growth.

Experimental condition was the same as that in Fig. 1.

- A. Curve 1, acetate; curve 2, succinate; curve 3, formate; curve 4, lactate; curve 5, L-glutamate; curve 6, DL-alanine. B. Curve 7, L-leucine; curve 8, M/250 L-tyrosine; curve 9, M/500 catechol; curve 10, DL-phenylalanine; curve 11, M/200 pyrogallol.

(3) Comparison among cells grown at different temperatures.

a) Oxidative activities.

Comparison was made among oxidative activities of bacterial cells grown at different temperatures (10° and 30°). At 10° , *Ps. aeruginosa* was able to grow only very slowly on 1 per cent peptone-broth agar and showed a 3 day-long latent period, the maximum growth yield being about half that at 30° . After 7 days of culture the organism reached the stationary phase of growth. The bacterial cells were collected at the initial, exponential and stationary phases of growth, and the oxidative activities were compared with those of the control in each corresponding phase of growth at 30° (Table 6). As shown in Table 6A, the capacity for oxidation of aromatic substrates was enhanced with the progress of growth, while the ability for oxidation of L-glutamate decreased. The oxidative activities of the cells grown at 30° were generally higher than those of the cells grown at 10° ; here, there was no marked increase in activity with time of culture (Table 6B). These results indicate that the growth at low temperature favours the synthesis of the ring-oxidizing system in the cells.

b) Effect of inhibitors.

The effect of various inhibitors on the catechol-oxidizing activity of the cells grown at different temperatures (10° and 30°) is shown in Table 7.

The effects of some of the inhibitors were slightly different according to the

Table 6. Comparison among oxidative activities of *Ps. aeruginosa* grown at different temperatures (10° and 30°).

Experimental condition was the same as that in Fig. 1. Final concentrations of substrates were M/250 of L-tyrosine, M/500 of catechol, M/250 of pyrogallol, M/30 of DL-phenylalanine and M/30 of L-glutamate. Reaction temperature, 30°. 3, 5, 7 and 10 days in A as well as 2, 8, 15 and 18 hours in B correspond to the initial, exponential, early stationary and late stationary phases of growth in each culture, respectively.

A, Cells grown at 10°; B, Cells grown at 30°.

Qo₂

Substrate	Time of culture (days)			
	3	5	7	10
A L-Tyrosine	16	21	32	36
A Catechol	22	32	56	66
Pyrogallol	20	28	29	24
DL-Phenylalanine	12	25	24	28
L-Glutamate	107	95	76	76
Substrate	Time of culture (hours)			
	2	8	15	18
B L-Tyrosine	—	52	41	59
B Catechol	—	24	35	38
Pyrogallol	—	30	15	17
DL-Phenylalanine	—	30	30	35
L-Glutamate	—	130	130	135

Table 7. Effect of inhibitors on catechol oxidizing activity of cells (stationary phase) grown at different temperatures (10° and 30°).

The reaction mixture was the same as that in Fig. 1. Reaction temperature, 30°; catechol, M/500. Final concentration of inhibitors was always 0.001 M. Numerals indicate the percentage of inhibition to the control.

Inhibitors	Growth temperature	
	10	30
8-Hydroxyquinoline	40	40
Diethyl-dithiocarbamate	52	55
KCN	58	38
Azide	12	15

growth temperature. The inhibition by cyanide was much higher in the 10° culture (about 60 per cent) than in the 30° culture (about 40 per cent). Other reagents caused nearly the same inhibition in both cultures.

c) Heat stability of cells.

Finally, the heat stability of the catechol oxidizing system was examined. The cells grown at 10°, 24° and 30° were collected at their respective stationary phases

of growth and treated for 10 min. at 40° or 50°; then, the oxidation of catechol by the treated cells was measured (Figs. 4 and 5). As shown in these figures, the higher the culture temperature, the greater was the resistivity of this enzyme system to high temperature.

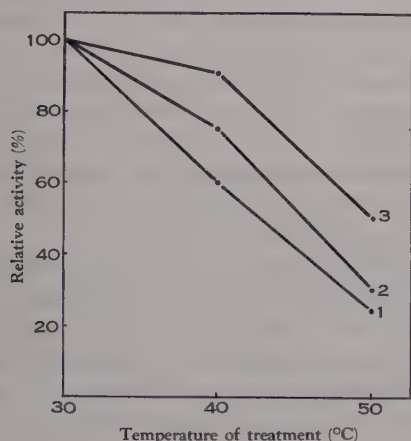


Fig. 4. Effect of heat treatment (40° and 50°) on catechol oxidizing activity of cells (stationary phase) grown at various temperatures (10°, 24° and 30°).

The reaction mixture was the same as that shown in Fig. 1. Reaction temperature, 30°; Catechol M/500.

Curve 1, cells grown at 10° for 10 days; curve 2, cells grown at 24° for 24 hrs.; curve 3, cells grown at 30° for 18 hrs.

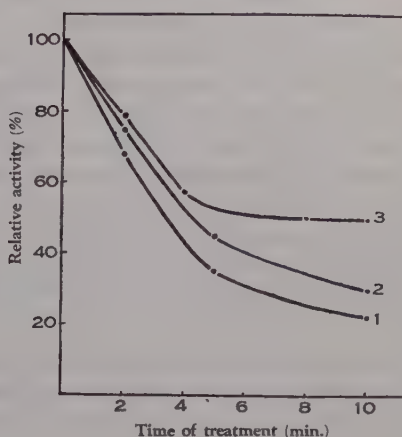


Fig. 5. Time-course of change in catechol oxidizing activity at 50° of cells (stationary phase) grown at various temperatures (10°, 24° and 30°).

The reaction mixture was the same as that in Fig. 1. Reaction temperatures, 30°; Catechol M/500.

Curve 1, cells grown at 10° for 10 days; curve 2, cells grown at 24° for 24 hrs.; curve 3, cells grown at 30° for 18 hrs.

Discussion

In the above-described experiments, it was shown that the effects of low temperature treatment on the bacterial capacity for oxidation change with the growth of the organism (Figs. 1, 2 and 3). In fact, the effect of low temperature-treatment on the oxidative activity of the cells in the initial growth phase was remarkably different from that in the exponential or the stationary phase. In the case of the cells in the initial phase, the activity of the oxidation of organic acids, except formate, after decreasing for a time, gradually recovered the original level. The oxidation of amino acids was, however, little affected. On the other hand, the oxidation of organic acids and amino acids by cells in the exponential or the stationary phase decreased gradually, never regaining the activity level of the untreated cells. On low temperature treatment of the cells in the initial phase of growth, it is probable that the enzymes of the terminal oxidase system are affected. But in the cells at other growth phases, both the dehydrogenases and the terminal oxidase system seem to be simultaneously affected.

It will be natural to assume that the cells in the initial phase of growth are different in their metabolic pattern from those in the later phases of growth. Recently, in the studies on the respiratory enzyme system of *Bacillus cereus*⁸), it has also been

shown that the status of the respiratory enzyme system in the cell changes with the growth stages. Thus the respiratory enzymes were suggested to exist in a soluble state in the early stage of growth and later become combined with cell particles. It is possible that low temperatures as examined in the present study cause a physical change with respect to soluble vs. particulate state of the cellular enzyme systems.

The effect of low temperature on the oxidation of aromatic substances by this bacterium differed from the above-stated in that the oxidation of these substances was not inhibited by low temperature treatment, irrespective of the growth phase of the cells. Moreover, in cells grown at 10°, the oxidation was found to be enhanced to some extent with time of culture, suggesting that the low temperature accelerated the synthesis of the enzyme systems responsible for the oxidation of aromatic substances. It has also been reported that low temperature is favourable to the formation of tyrosinase in microorganisms, insects and plants^{4, 9, 10}).

As shown in Table 7, the effect of inhibitors on the enhanced catechol oxidation in cells grown at 10° was not very significantly different from that observed in those grown at 30°. Figs. 4 and 5 show that when the culture temperature was lower, the catechol oxidative system was more susceptible to high temperature treatment (40°–50°). In *Pseudomonas*, catechol is known to be metabolized by pyrocatechase¹¹) and it may be assumed that the action of low temperature is favourable for this enzyme system.

Summary

Effect of low temperature treatment on oxidation in *Ps. aeruginosa* at different growth stages was studied. It was shown that the susceptibility to low temperature treatment of the cellular oxidation system in the initial phase of growth is different from that of cells in the exponential or the stationary phase. In the initial phase of growth, the oxidation of various organic acids, except formate, is at the lowest level at about 2–3 days of low temperature treatment, but oxidations of amino acids and aromatic substances are little affected by low temperature. This decrease in activity of organic acid oxidation is not attributable to the decrease in dehydrogenase activities. In other phases of growth, the activity of oxidation of organic acids and amino acids gradually decreases with time of low temperature treatment, owing to the decrease in activity both of dehydrogenase and terminal oxidase systems. The oxidation of aromatic substances is stable to low temperature treatment. Low growth temperature favours the synthesis of the phenol-oxidizing system.

These experiments were performed in the laboratory of plant physiology, Faculty of Science, Hokkaido University.

The writer acknowledges the helpful suggestion and criticism of Professor Shochiro Usami during the course of this work. She would also like to thank the members of the Department of Botany, Faculty of Science, Hokkaido University, for valuable discussion.

References

- 1) Gale, E.F., Bacteriol. Rev. **7**: 193 (1943).
- 2) Sasaki, S., and Usami, S., Sym. Enzyme Chem. Japan **7**: 61 (1952).
- 3) Usami, S., Kurabayashi, M., Masubuchi, N., and Teraoka, H., Biol. Sci. (Tokyo) **6**: 44 (1955).
- 4) Horowitz, N.H., and Shen, San-chuin, J. Biol. Chem. **197**: 513 (1952).
- 5) Minsky, M.J., and Stokes, J.L., J. Bact. **64**: 337 (1952).
- 6) Maruyama,

- Y., and Mitui, H., J. Biochem. **45**: 169 (1958). 7) Yamaguchi, S., Acta Phytochim. **8**: 157 (1934). 8) Nakada, D., Matsushiro, A., Kondo, M., Suga, A., and Konishi, K., Med. J. Osaka Univ. **7**: 809 (1957). 9) Westergaard, M., and Hirsch, H., Proc. 7th Symp. Colston Research Soc. 171 (1954). 10) Jevitt, J., and Todd, G.W., Physiol. Plantarum **5**: 419 (1952). 11) Hayaishi, O., and Hoshimoto, Z., Med. J. Osaka Univ. **2**: 33 (1950).

摘 要

仲 尾 澄 子: 細菌の酸化におよぼす低温の影響

種々の生長期にある *Pseudomonas aeruginosa* の酸化作用におよぼす低温の影響をしらべた。

生長期初期においては、低温に対する感受性は、他の生長期のそれと非常に違っている。すなわち、初期では有機酸の酸化が、低温（約 5°）処理の 3 日ぐらいで最低に達するが、アミノ酸やフェノール物質の酸化は、低温の影響を受けない。また有機酸の脱水素酵素系は、この処理によって減少しない。他の生長期、すなわち exponential および stationary の時期では、有機酸、アミノ酸の酸化は、低温処理の日数の増大とともにしだいに減少する。この時、脱水素酵素系も末端酸化系もともに減少する。フェノール物質の酸化は低温の影響を受けない。

菌を低温（10°）で培養する時は、フェノール物質の酸化は増大する。フェノール酸化系は、低温に対して安定であるのみでなく、低温条件は *Ps. aeruginosa* のフェノール酸化系の合成に有利に働くのではないかと思われる。（福島県立医科大学薬理学教室）

Leaf Growth as Influenced by Dry Matter Production*

by Toshiro SAEKI**

Received July 29, 1960

Yield ultimately depends upon the efficiency of the photosynthetic processes and upon the extent of the photosynthetic surface¹). Recent investigations have revealed that these two components of growth are not necessarily independent of each other, for example, a thick development of foliage in a plant community results in decrease of photosynthetic efficiency^{2, 3}). On the other hand, it is quite natural that growth of leaves is affected by the photosynthetic rate, because major constituents of leaves originate from photosynthate. The importance of this fact has, however, often been overlooked and thereby some erroneous conclusions have been drawn. For example, Milthorpe, referring Ashby and Wangermann's results⁴), stated that the difference in final size of leaves developed at different seasons was owing mainly to the influence of environment on cell division, because the number of cells per leaf was influenced by season while final cell size was much the same regardless of season⁵). The same size of cell, however, cannot be realized without supply of different amount of matter corresponding to the different number of cells. This different amount of matter is none other than a resultant of different effects of environmental conditions upon the dry matter production.

This paper is primarily concerned with dry matter production as an essential determinant of leaf growth. Moreover, a further attempt will be made to adopt the obtained results to the interpretation of the vertical distribution of leaves in a plant community.

Relation between growth and matter production in individual leaves of a plant

Younger growing leaves of herbaceous plants are always situated near the shoot apex, and the growing region shifts upwards with the proceeding stage of growth. In order to visualize the upward shift of the growing region the author determined dry weights of individual leaves at every insertion at intervals of two or three days using green gram plants (*Phaseolus viridissimus*) as material. The seeds were sown in square disposition at a spacing of 10 cm. in a plot at the Experimental Farm of the Tokyo University of Education. After germination thinnings of population were made so frequently that no mutual shading occurred. Changing features of photosynthetic capacity in individual leaves of this material plant have already been described in a previous paper⁶). For dry-weight determination of leaves and other organs, 15–20 sample plants were taken every sampling occasion. The results of leaf-weight growth at every node of stem are summarized in Fig. 1-a. With progress of time the most vigorously expanding region removed upward along the stem. This by no means implies that mature leaves have ceased matter production. Young leaves owe at least a part of their constituents and formative energy to the substances produced in mature leaves. The quantitative estimate of this bearing was done by calculating the amount of photosynthetic product for four days (2 to 6, Sept.), on the

* Supported by a Grant in Aid of Scientific Research of the Ministry of Education.

** Department of Botany, Faculty of Science, University of Tokyo, Hongo, Tokyo, Japan.

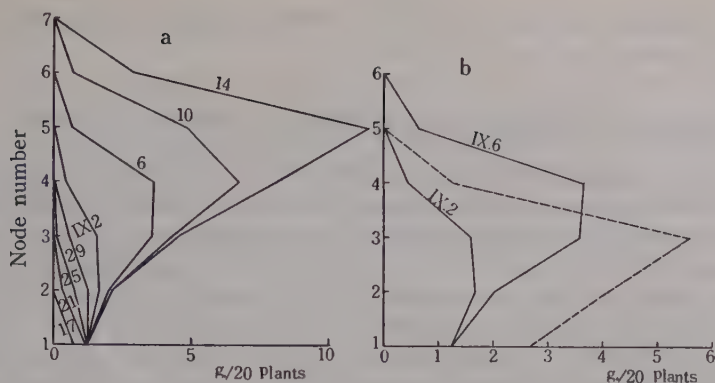


Fig. 1. a: Change of mean dry weight of individual leaf at each node of a green gram plant. Nodes are numbered from the first foliage leaf. The numbers at the curves indicate the date, from 17 Aug. to 14 Sept. b: Relationship between calculated amount of photosynthesis in $C_6H_{10}O_5$ (broken line) and realized dry weight increment (solid line-IX.6), in individual leaves.

basis of the photosynthetic capacity reported in a previous paper⁶⁾ and of the weather conditions at corresponding stage, particularly the diurnal change of radiation. The results of the calculation were shown in Fig. 1-b. It is evident that the broken line indicating the amount of photosynthetic product runs quite differently from the solid line showing realized growth in weight of each leaf; the first to third leaves (numbered from the base) must have supplied their photosynthate to the younger leaves, and the fourth to sixth leaves thereby increased their weight more than they could by themselves.

We may, therefore, assume some competition among successive leaves as well as different organs for acquiring photosynthetic product. The competition among organs should determine the 'distribution ratio'⁷⁾ in photosynthetic product among respective organs. Similarly the competition among growing leaves should determine the 'distribution ratio' in photosynthetic product among respective leaves. Fig. 2 shows such 'distribution ratio' calculated from the daily growth of individual leaves of green gram plants. A noticeable fact that can be drawn from Fig. 2 is that the shape of the time trend of the 'distribution ratio' was roughly symmetric and not much different among the successive leaves, despite the fact that their final weights diverged very widely. The case is not altered, if leaf growth is expressed on an areal basis in stead of a dry weight basis. As inevitable deviations involved in the sampling

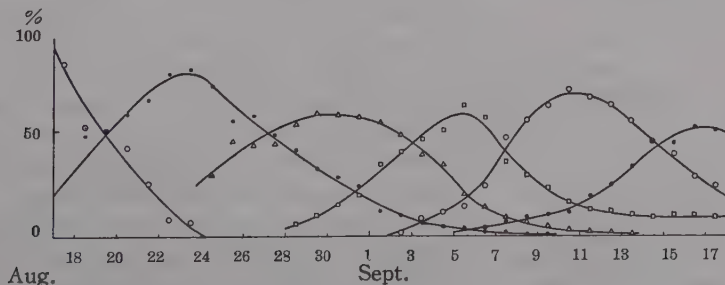


Fig. 2. Distributional pattern of photosynthetic product in successive leaves of green gram plants.

prevent the generalization of this finding; a further precise experimentation should be performed to present more reliable evidence.

Distribution of product among successive leaves of a plant

The following experiments were designed; tobacco plants (*Nicotiana tabacum* L. 'BrightYellow') were grown in wooden boxes (32×32 cm. and depth 9 cm.) within a light- and temperature-controlled growth cabinet from 14 February to 5 May, 1958. Ten fluorescent lamps (40 W, Mazda-white) as light source continuously illuminated material plants throughout the experiment. At the beginning of the experiment the illumination at the soil surface was 6000 lux. The temperature within the cabinet was regulated to $25^{\circ}\pm 1^{\circ}$. These conditions ensured apparently sound growth of tobacco plants without excessive stem elongation. After germination, seedlings with cotyledons were transplanted from a petri-dish to the wooden boxes filled with loamy soil, in two modes of planting—single and dense plantings. In the former only three seedlings were planted at about the centre of a box, and after rooting one healthy seedling was left, the other two being eliminated. In the case of dense planting the seedlings were planted two each at 5×5 positions, in square disposition with a constant interval of 6 cm., and after rooting only one of the two was left behind at every position in order to make the population uniform. Every other day each box was sprayed with one litre of Boysen Jensen's culture solution at a 1/5-concentration.

Leaf area (S) was assessed by measuring two measures of lamina; the one is the widest width of lamina (a), the other the length of lamina (b), i.e. from the tip to the base of the lamina. The latter was decided in a large leaf with vague basal point, as an intersection of extensions of both inside-concave periphery lines. The area S estimated as an ellipse by applying $S=\pi/4\cdot ab$ is compared with actual area measured by a planimeter in Fig. 3, being plotted in logarithmic scale, to admit a wide range of data. In the range of area so far measured, the estimated area was proportional to the actual area (0.93:1), because the plots fell along a straight line

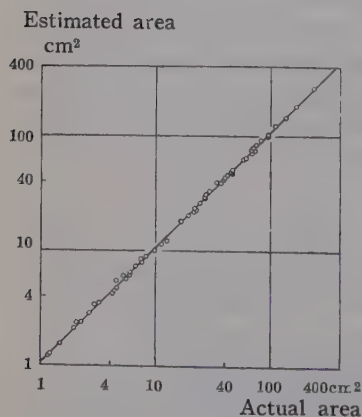


Fig. 3. Leaf area estimated as an ellipse in relation to actual leaf area.

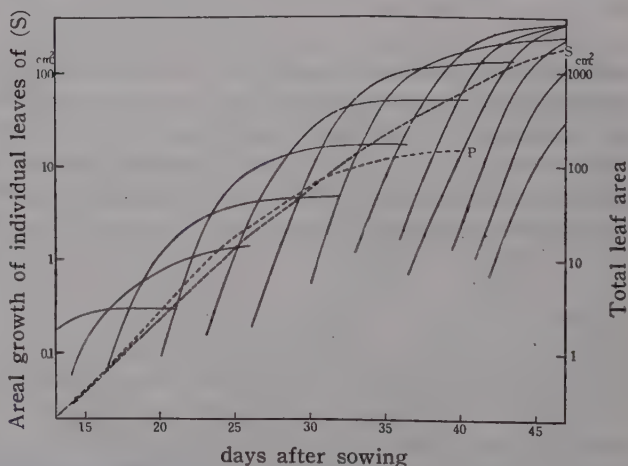
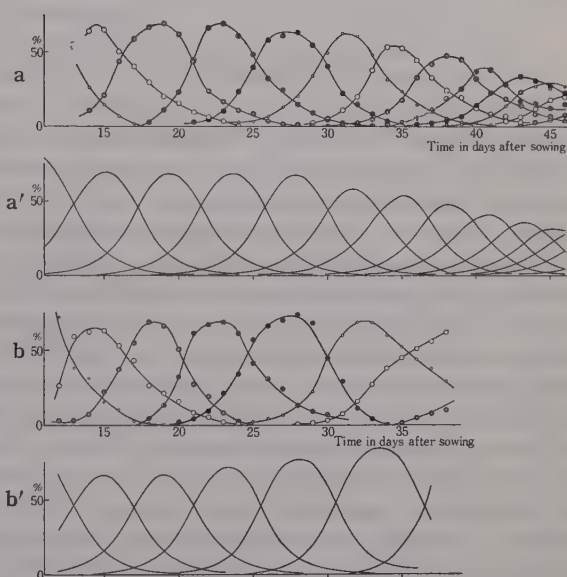


Fig. 4. Solid lines: Growth curves of leaves at successive stem nodes of a tobacco plant cultivated solitarily. Broken lines: total leaf area of the experimental plants. S, a plant grown solitarily; P, a representative plant from the dense planting.

having a gradient of 45° with small deviations, irrespective of the lanceolate shape of larger leaves.

Developmental courses of successive leaves shown in Fig. 4 are of identical nature with those obtained by Milthorpe⁶⁾, Bünning and Konder⁸⁾, *et al.* The singly-grown plant increased its total leaf area almost exponentially up to four weeks after transplanting, while the centrally-placed plant in the dense population, the representative of an individual in a plant community, ceased the exponential increase after about three weeks. This growth retardation was undoubtedly caused by the overshadowing of the neighbouring plants.

Fig. 5. Distributional pattern of photosynthetic product in successive individual leaves of tobacco plants cultivated under the condition of constant illumination and temperature. The first curve is for cotyledons, the next, for the first leaf, and so on. a, observed in a solitary plant; b, observed in a plant from the dense planting; a', calculated for the solitary plant; b', calculated for the plant from the dense planting.



In Fig. 5-a, b, is shown the percentage of daily area-increment of successive leaves in relation to the total increment of leaf area. The curves are quite similar to the results obtained in the foregoing field experiment with green grams. The first to fifth leaves gave the same shape of time trend. From the sixth leaf onward the maxima of the curves became smaller and smaller in the solitary plant together with accelerated leaf appearance, while reverse was the case in the populated plant. Apart from these, a remarkable point is that the shape of every curve did not lose its symmetry notwithstanding a great diversity in final leaf areas among the levels of insertion and between both sorts of planting. Dry weight per area of leaf increased to some extent with progress of node number, and the increase was notably steep at small top leaves. Nevertheless, when leaf growth is expressed on a weight basis instead of areal basis, the finding obtained here still persists.

These characteristic growth patterns have lead to a hypothesis that the internal force of every leaf to attract raw materials for its own growth follows the equal shape of time trend, like the 'normal probability curve' or 'differentiated sigmoid curve'. According to this hypothesis, changes of form of the distribution curves must be merely a natural consequence either of accelerated or retarded leaf appearance. This can be proved by testing whether the experimentally obtained distribution curves (Fig. 5-a, b) will reappear, when this hypothesis is reversely employed in the theoretical calculation of 'distribution ratio among successive leaves'. This

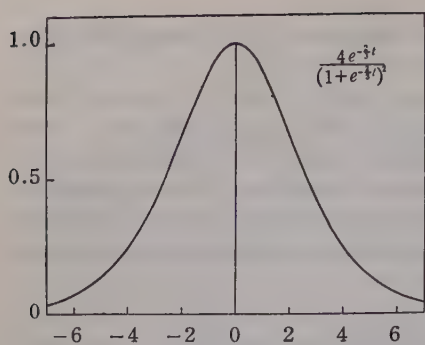


Fig. 6. Differentiated sigmoid curve employed for obtaining the curves of Fig. 5-a', b'.

procedure was practised by using one and the same 'differentiated sigmoid curve' as shown in Fig. 6 for every leaf. The theoretical curves which were induced by combining this original curve with the actually determined plastochrone were provided in Fig. 5-a', b', for the solitary plant and populated plant, respectively. We can see that the curves obtained are quite similar to the experimental ones in both cases, in other words, the introduced hypothesis could explain the experimental results without any contradiction.

According to Avery⁹⁾ cell division in tobacco leaves enters into cessation when leaf area reaches 1/6 to 1/5 of its final size and further expansion of leaves is caused solely by cell enlargement. Most recently, however, Sunderland¹⁰⁾ has followed the developmental changes in volume and number of leaf cells of *Helianthus annuus* and *Lupinus albus* by a revised technique and come to a conclusion that cell division goes on for a considerable period of time and further leaf expansion after cessation of cell division is comparatively small. The time trend of increase in cell number of an individual leaf corresponded closely to symmetrical curve. The frequency of cell division in the leaf, therefore, seems to be reflected in the characteristic symmetry of the material-attracting power mentioned above. Further, he stated in his discussion that the duration of cell division of sunflower leaves is much longer in the tenth leaf than in the second paired leaves. This is inconsistent with the hypothesis in this paper where the identity of the duration of every leaf growth is assumed. Inspection of his data, however, clarifies that the period from initiation to cessation of cell division is 66 days for the second pair of leaves (Fig. 2 in his paper) and 74 days for the tenth leaf (Fig. 3 in his paper). The difference of 12% between these periods is very small if compared with the differences, 450% in cell number, 180% in leaf area and 190% in fresh weight of leaf. We can draw, therefore, rather a reverse conclusion that duration of cell division is not much different between the two sorts of leaves. Ashby and Wangermann⁴⁾ indicated that with *Ipomoea purpurea* the duration of growth of individual leaves did not vary much and showed no trend either with level of leaf insertion or date of sowing.

Cause of difference in plastochrone

Then occurs there a question as to the cause of difference of plastochrone or leaf production. In the original sense 'plastochrone' denotes the time interval between differentiation of successive leaf primordia, yet they are very small and hidden from the naked eye, and we cannot easily determine their growth inception. As plastochrone, therefore, the author conveniently determined the time intervals between the maxima of the curves showing the time trend of the 'distribution ratio' between successive leaves. The difference in plastochrone as produced by the two sorts of planting, if any, must be ascribed to the difference either of external or of internal factors of plant. In the experiment with tobacco plants there was no difference in the general environmental conditions. Although in the dense planting the lower leaves were suffering from a considerable depression of illumination, the stem apex and upper leaves were accepting full illumination. In addition, the solitary plant was

accepting the same intensity of light throughout growth, yet the plastochrone decreased with growth of the plant. Thus the direct effect of light fails to interpret the changes in plastochrone. On the other hand, one of the internal factors to be likely responsible for the changing plastochrone is the amount of dry matter production itself. It is highly presumable that high production stimulates a plant to accelerate leaf emergence or growth inception of leaf primordia, while low production depresses it. Being favoured with sufficient light intensity, solitary plant progressively increases its matter production with expanding total leaf area. If plastochrone were constant, the amount of photosynthetic product would, in a short time, exceed the protein-synthetic capacity of the expanding leaves.

Productivity as a limiting factor of leaf growth

Leaf growth has been an object of interest on the view of hormonal control. Adenine¹¹⁾, kinetine¹²⁾, coumarine¹³⁾ and some inorganic metals¹⁴⁾ have been recognized to promote expansion of leaf disks or etiolated leaves of some species. No evidence has, however, been presented hitherto such hormone-like substances were restricting leaf growth of a healthy plant under natural conditions. Then, what can be the cause of difference in size between successive leaves? One may ascribe it to the genotypical characters of the species, but it is obvious that, by varying environmental conditions, a large variety of leaf area can be produced even at the same insertion. In the analyses of the growth response of leaves to the environmental conditions, many investigators have recognized concerning leaf growth rate two separate components, the rate of cell division and that of cell enlargement. These two attributes are, however, in reality not independent of each other; in the case of the same level of dry matter productivity, high rate of cell division must of necessity result in small cell size. It is therefore rather reasonable to separate the factors of leaf growth rate into cell division and dry matter productivity instead of cell enlargement.

From the experimental results described above, it follows that in vigorously growing plants the amount of raw materials for synthesis of protein, cellulose, etc. can limit leaf growth. In this case the dry matter productivity and the competitions both between organs (leaves, stems, roots etc.) and among individual growing leaves determine the growth rate of each leaf. In other words, final leaf size depends upon the magnitudes, at the time when the leaf expands, of the following three major factors; 1, dry matter productivity of the whole plant; 2, proportion of the total foliage growth to the total dry matter production; 3, plastochrone. The first and the second determine the total leaf growth of the whole plant and the third determines the amount of material incorporated in each of the expanding leaves. It is fairly familiar that the widespread technique of 'topping' to accelerate the growth of lateral shoots also causes appreciable expansion of the remaining leaves. This is a natural consequence of decreased number of expanding leaves, i.e. of weak competition among them. In the same way, in plants projecting lateral shoots, leaf production occurs at several growth centres and consequently, many small, but rather uniform, leaves result.

If the time trend of material-attracting power is expressed by an identical curve common to every leaf, the proportion of distribution of produced matter into individual leaf varies again along a curve which is similar to the original curve but the maximum of which depends on the number of expanding leaves and, accordingly, on plastochrone. As daily weight increment of a leaf is the product of the two factors—daily increment in the total leaf weight (ΔF) and distribution ratio of photosynthetic product in this leaf (D)—the size of n -th leaf (L_n) at t days after growth inception of the 1st

leaf will be shown in the following formula;

$$L_n = \sum_0^t \Delta F \cdot D = \sum_0^t \Delta F \frac{4H \cdot \exp(-\lambda T)}{\{1 + \exp(-\lambda T)\}^2} \quad (1)$$

$$[T = t - (n-1)\tau - A]$$

where λ is a constant, and A is regarded practically as a constant for all leaves, because it stands for the time interval from the inception of growth of each primordium to the stage possessing the maximum 'distribution ratio'. So far as plastochrone τ is constant, the maximum ratio of distribution of photosynthetic product into each leaf, H , is also constant. The smaller the value of τ , the smaller the value of H , and *vice versa*. The interrelation can be expressed by the following formula and was graphically shown in Fig. 7.

$$\frac{1}{H} = \sum_{m=1}^{\infty} \frac{8 \exp(-m\lambda\tau)}{\{1 + \exp(-m\lambda\tau)\}^2} + 1 \quad (2)$$

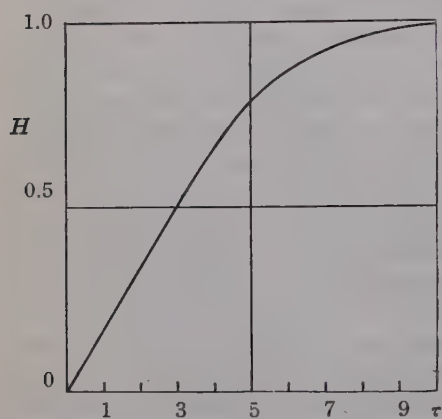


Fig. 7. Maximum distribution ratio H as related with plastochrone τ . Calculated from Equation (2).

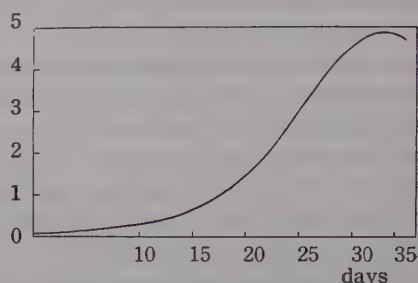


Fig. 8. Daily increase of leaf area index in a plant community. Method of calculation is shown in the text. $F_0=0.04$, $\varepsilon=0.5$ and $\delta=30$ g./sq. m.

Theoretical course of leaf growth

Daily weight increment (ΔF) in the whole leaf of a plant originates in the daily surplus production (P) produced by the foliage. P can be formulated as a function of leaf area index ($\text{LAI}=\bar{F}$) and light intensity, as seen in a previous paper¹⁵). Therefore, if proportion (ε), in which a part of surplus production is synthesized into the constituents of expanding leaves, and conversion constant (δ) from leaf area to leaf dry weight ('leaf dry matter index' after Totsuka and Monsi¹⁶), are given beforehand, the time course of the magnitude of ΔF (or $\Delta \bar{F}$) should easily be calculated. The procedure will be interpreted as follows: Let initial LAI be \bar{F}_0 . From the curve showing \bar{F} -daily surplus production relationship (see Fig. 5 in a previous paper¹⁵)) one can obtain the surplus production P_0 at \bar{F}_0 , hence in the following day dry weight increment of the whole leaves $\Delta F = \varepsilon P_0$, and total dry weight of leaves per unit ground area of the plant community, $F_1 = F_0 + \varepsilon P_0$. The surplus production at $\bar{F}_1 (= F_1/\delta)$, P_1 , is read again from the said figure and so on. Fig. 8 gives a typical change of $\Delta \bar{F}$ obtained through such procedure. According to this figure and Equations (1) and (2), one can now obtain the time course of growth of each individual

leaf. One of such examples is illustrated on the system of node number-leaf area-coordinates in Fig. 9. The analyses made in the present paper find sufficient justification in the fact that the curves in this figure are quite of the same nature as in Fig. 1-a. Furthermore, it is quite natural that the curves presented in Fig. 9 resemble the vertical distribution of photosynthetic system in a plant community²⁾, because the numerical order of leaf insertion runs roughly parallel with the height of each leaf, and a plant community is made up from individual plants. In this respect details will appear in the following paper.

Summary

1. The growth courses of individual leaves were pursued in green gram plants under field conditions, and in tobacco plants under continuous illumination and at constant temperature of a growth cabinet.

2. In each of successive leaves on the main stem of a green gram plant, comparison was made between the amount of dry matter produced by photosynthesis and the amount of dry weight increased during the same time interval (Fig. 1-b). The comparison indicates that for the growth of younger leaves supplementary material must be imported from older leaves.

3. It was postulated that an expanding leaf has an 'internal force' whereby the leaf attracts raw materials for its own growth. The curve that shows the time trend of the internal force resembles the 'normal probability curve', and is quite of the same shape for every leaf along the main stem.

4. In the tobacco plants as exposed to continuous illumination, constant temperature and sufficient nutrient condition, plastochrone appears to be influenced only by dry matter production of the whole plant.

5. Final leaf size depends upon the magnitudes, at the time when the leaf expands, of the following three major factors: i) dry matter productivity of the whole plant; ii) proportion of the total foliage growth in dry matter to the total dry matter production; iii) plastochrone. The first and the second determine the absolute quantity of dry matter in total foliage growth of the whole plant, and the third determines the amount of dry matter incorporated into each of the expanding leaves.

6. In plants devoid of branching, individual leaf size at any insertion at any time can be given in Equation (1), if daily increase of the total leaf amount (ΔF) or of the total leaf area ($\Delta \bar{F}$) is given beforehand. The change of $\Delta \bar{F}$ with time is shown in Fig. 8, which was drawn on the basis of the data in a previous paper¹⁵⁾, and the leaf amounts at different days were calculated thereby from Equation (1), the result being illustrated in Fig. 9.

The author wishes to express his cardinal thanks to Prof. M. Monsi for his invaluable advice.

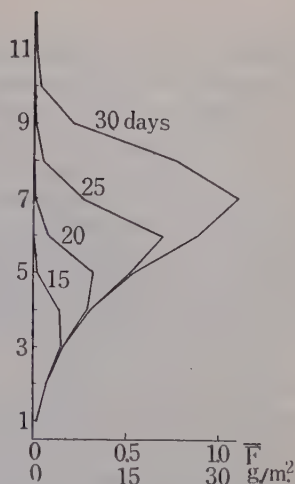


Fig. 9. Calculated leaf amounts at different nodes and at different days. Calculation is based on Figs. 7, 8 and Equation (1).

References

- 1) Watson, D. J., *Advances in Agronomy* **4**: 101 (1952). 2) Monsi, M., and Saeki, T., *Jap. J. Bot.* **14**: 22 (1953). 3) Watson, D. J., *Growth of Leaves*, London 178 (1956). 4) Ashby, E., and Wangermann, E., *New Phytol.* **49**: 23 (1950). 5) Milthorpe, F. L., *Growth of Leaves*, London 141 (1956). 6) Saeki, T., *Bot. Mag. Tokyo* **72**: 404 (1959). 7) Iwaki, H., *Jap. J. Bot.* **16**: 210 (1958). 8) Bünning, E., and Konder, M., *Planta* **44**: 9 (1954). 9) Avery, G. S., Jr., *Am. J. Bot.* **20**: 565 (1933). 10) Sunderland, N., *J. Exp. Bot.* **11**: 68 (1960). 11) Miller, C. O., and Meyer, B. S., *Plant Physiol.*, **26**: 631 (1951). 12) Kuraishi, S., and Okumura, S., *Bot. Mag. Tokyo* **69**: 300 (1956). 13) Miller, D. M., and Haagen Smit, A. J., *Proc. Nat. Acad. Sci.* **25**: 184 (1939). 14) Miller, C. O., *Arch. Biochem. and Biophys.* **32**: 216 (1951). 15) Saeki, T., *Bot. Mag. Tokyo* **73**: 55 (1960). 16) Totsuka, T., and Monsi, M., *ibid.* **73**: 14 (1960).

摘 要

佐伯敏郎； 葉の生長におよぼす物質生産の影響

圃場でヤエナリを、また定温定照度下でタバコを栽培して個々の葉の生長経過を追跡した。実測された個々の葉の乾量増加は光合成の値を使って算出された乾量の増加とは一致しない (図 1-b)。すなわち若葉は老葉の光合成生産物の一部をとって自己の生産以上に生長する。そこで全光合成生産物が一つの植物のすべての伸長葉の間に分配された割合を調べてみた。その結果葉位により個々の葉の最終の大きさには非常に大きな差があるにもかかわらず、この分配率の時間的变化は各葉位の葉の間で大差なく確率曲線の形に類似した推移をする (図 2, 5a, b,)。ただ出葉速度が大きくなれば分配率の最大が小さくなり、出葉速度が小さくなればそれが大きくなる。この現象はつぎのような仮説によって説明できる。すなわちおのおのの伸長葉には光合成生産物質を吸引する力があって、これがどの葉でも原基の生長開始とともに一定の時間的变化をたどり、分配率の変化は各伸長葉間の物質吸引力の比によりきまる。したがって個々の葉の最終の葉面積は葉の伸長時におけるつぎの3つの量によりきまることになる。1) 全物質生産力、2) その生産物中全葉の生長量となる割合(ϵ)。3) 出葉の時間的間隔 (plastochrone)。この中1と2で全葉の生長量がきまり、3によって個々の葉の生長量がきまる。群落状態の植物について、前報¹⁾における葉面積指数一日物質生産量の関係と、葉乾量対葉面積比 (leaf dry matter index— δ) とを用いれば、一日当たりの全葉の生長量の変化が8図のように計算できる。次に(1), (2)式を用いて任意の葉位における任意の時間の葉の量を計算した結果 (図 9)、実測された葉位と葉乾量の関係を示す 1-a 図と同じ性質のものがえられた。(東京大学理学部植物学教室)

On *Spirillum putridiconchylum* nov. sp.*

by Yasuke TERASAKI**

Received July 29, 1960

Since the term of spirillum was originated by Ehrenberg^{1,2)}, many organisms were described under the genus *Spirillum* by some investigators. However, the descriptions of the species reported prior to Giesberger³⁾ are so incomplete that they cannot be employed for the diagnosis of species. Giesberger's investigation on the genus *Spirillum* is most detailed and so important for the research in this part. According to him, the diagnosis of the genus *Spirillum* is as follows: "Spiralförmig gewundene, starre Zellen, welche beweglich sind mittels bipolarer Geisselbündel. Das Vermögen zur Endosporenbildung fehlt. Gram-negativ. Katalase positiv. Chemo-heterotroph; oxydieren verschiedene organische Substanzen, vorzugsweise Salze organischer Säuren. Im allgemeinen tritt Volutin als Reservestoff auf." On the basis of his own data and a thorough review of the literatures, he has differentiated nine species: *Spirillum undula* (Müller) Ehrenberg, *Sp. serpens* (Müller) Winter, *Sp. volutans* Ehrenberg, *Sp. tenue* (Müller) Ehrenberg, *Sp. minus* Carter, *Sp. kutsheri* Migula, *Sp. virginianum* Dimitroff, *Sp. cardinopyrogenes* Sardjito and *Sp. itersonii* nov. sp. Lately Cayton and Preston⁴⁾ isolated a new species of *Spirillum*, *Sp. mancuniense*. The description of this genus in Bergey's Manual of Determinative Bacteriology⁵⁾ is based on the monograph by Giesberger³⁾. It, however, differs from the description in Giesberger's in the retention of *Sp. lipoferum* Beijerinck and in the exclusion of *Sp. cardinopyrogenes*, Williams *et al.*⁶⁾ have isolated many species of *Spirillum* and have described the following ten new species and two new varieties of *Spirillum*: *Sp. linum*, *Sp. lunatum*, *Sp. curvatum*, *Sp. polymorphum*, *Sp. anulus*, *Sp. giesbergeri*, *Sp. beijerinckii*, *Sp. atlanticum*, *Sp. graniferum*, *Sp. sinuosum*, *Sp. serpens* var. *azotum* and *Sp. itersonii* var. *vilugatum*. In the same paper, they have proposed a key to the species of the genus *Spirillum* based on their own data and previous work. More lately Watanabe⁷⁾ isolated four new halophilic species of *Spirillum* from some marine shell fishes: *Sp. japonicum*, *Sp. halophilum*, *Sp. maritimum* and *Sp. minitrum*.

The present author succeeded in isolating a new species which could be classified under the genus *Spirillum* from a fresh water snail, *Semisulcospira bensoni* (Philippi) which had putrefied in his laboratory. The spirillum has been purely cultivated in vitro for about two years. This paper deals with the isolation and with the morphological, cultural and physiological characteristics of the spirillum.

Isolation

Source of materials: *Semisulcospira bensoni* (Philippi) was collected, in August 1958, at the same place as in previous paper⁸⁾ reporting a new species of *Cristispira* living in the crystalline style. Microscopic observation of the culture medium containing the putrid shell fishes revealed a large number of various spiral organisms moving around together with other microorganisms. These spiral organisms were preliminarily cultured in a Petri dish full of sodium chloride solution (0.1%, w/v) containing the broken putrid shell fishes. Then the pure culture was made by using the solution

* A part of this study was presented at the Annual Meeting of the Chūgoku-Shikoku Branch of the Botanical Society of Japan, held at Matsue in May 1960.

** Biological laboratory, Suzugamine Women's College, Hiroshima, Japan.

for isolation which was composed of 5 g. peptone, 3 g. yeast extract, 1 g. sodium chloride, 200 ml. shell fish extract and 800 ml. water.

For preparation of the shell fish extract, 500 ml. of water was added to 250 g. of the broken shell fishes, and boiled over free flame for 20 min. Then the extract was filtered. For the preparation of solid media, agar powder was incorporated into the liquid media at the rate of 0.7% (w/v). The pH of the media was adjusted at 7.0 to 7.2 before sterilizing.

Method of isolation: The surface of five agar plates was successively streaked with one loopful of the putrid medium, and these plates were incubated at 20°. After several days of incubation, each colony grown on the plates was transferred into the tubes containing the liquid media. From these cultures, a new species of *Spirillum*, *Sp. putridiconchylum* nov. sp. was obtained. Subcultures were made by transferring to the shell fish extract media or to nutrient broth or agar.

Culture media and cultural condition for the identification of the spirillum

The nutrient broth employed was of the following composition: extract of beef, 5 g.; peptone, 3 g.; distilled water, 1000 ml. As the solid medium, a 0.7% (w/v) nutrient agar or a 12% (w/v) nutrient gelatin was prepared. The pH of these media was adjusted at 7.0 to 7.2, which was the optimum pH for the growth of the spirillum. For testing of carbon or nitrogen source for the growth of the spirillum, single carbon or nitrogen compound was added to the said media, after Williams' procedure⁶). The temperature of incubation was 35°, which was the optimum temperature for the growth of the spirillum. The growth rate was determined by turbidity measured with an Erma photoelectric photometer.

Description of *Spirillum putridiconchylum* nov. sp.

(1) Morphological characteristics

Vegetative cell: The spirillum grown in the nutrient broth after incubating for 24 hrs. at 35° has a spiral-, S- or vibrio-shaped body, with obtuse ends. It is 5 to 60 μ in length (many are 5 to 30 μ), and 0.8 to 1.0 μ in diameter. The waves are 1 to 7 in number (many are 1 to 4 in number). The wave length is 5 to 7 μ . The width of the wave is about 2 μ . Refractive granules are present or absent with different individuals. The spirillum moves to either direction with equal rapidity and facility, rotating counterclockwise through the medium. In old cultures, the vegetative cells predominate and the microcysts are not found. The spirillum obtained from a colony on the nutrient agar plate after incubating for 24 hrs. at 35° is shorter than that in the nutrient broth. Many show the vibrio- or the S-shaped form, and are 3 to 10 μ in length. But occasionally the spirillum reaches a length of 20 μ or more. The diameter of the body is almost the same as that of the organism grown in the nutrient broth. Concerning the refractive granules, no differences were observed between the spirillum grown on agar and that grown in broth. The spirilla begin to move as soon as they are transferred into the liquid media.

Stained cell: Both the spirillum grown in the nutrient broth and the spirillum obtained from the colony on the agar exhibit almost the same staining features. When the spirillum cultivated for 24 hrs. at 35° is stained with Löffler's methylene-blue, it shows somewhat different appearance, such as (1) the so-called transverse-barred or the chambered appearance in which some unstained portions are distributed

in the pale cytoplasm along its length, (2) the appearance which is uniformly colored blue without showing any structure, or (3) the appearance which shows one to several volutin granules, taking purplish-red color, scattered in the body dyed uniformly blue or revealing the transverse-barred appearance. The volutin granules are most abundantly found in the spirillum after incubating for 3 days. The flagella are readily stained by Löffler's method. Most of the organism in young cultures have one flagellum at each pole of the body. The flagella increase in number as the culture ages. The spirillum obtained from a colony incubated for two or more days has a tuft of several flagella. The spirillum in the nutrient broth has less flagella than the spirillum obtained from a colony on agar. The spirillum is Gram-negative.

(2) Cultural characteristics

Agar colonies: Growth is speedy. After incubating for 24 hrs. at 35°, surface colonies are punctiform or circular (0.8 to 1.2 mm. in diameter), smooth, entire, finely granular, low convex, creamy-white, opalescent, and have a ground-glass appearance when they are viewed with the hand lens with transmitted light. Deep colonies are smaller than the surface colonies, and are round, elliptical, spindle or irregular.

Gelatin colonies: Colonies are seen with the naked eye after 2 days at 20°. After incubating for 7 days at 20°, surface colonies become circular (1.0 to 1.2 mm. in diameter), smooth, undulate or lobate, coarsely granular, umbonate, brownish-white, opaque, and the centers of the colonies are brown when viewed with the hand lens. Deep colonies are similar to the surface colonies except that the size is small (0.5 to 0.8 mm. in diameter) and that the edge is undulate, lacerate or lobate.

Agar stroke: Growth is moderate, beaded, creamy-white, glistening and brittle. Fetid odor is absent, and the medium remains unchanged.

Agar stab: Growth is seen along the entire stab and on the surface, but scanty in the lower part of the stab. The line of puncture is papillate, and the medium is unchanged.

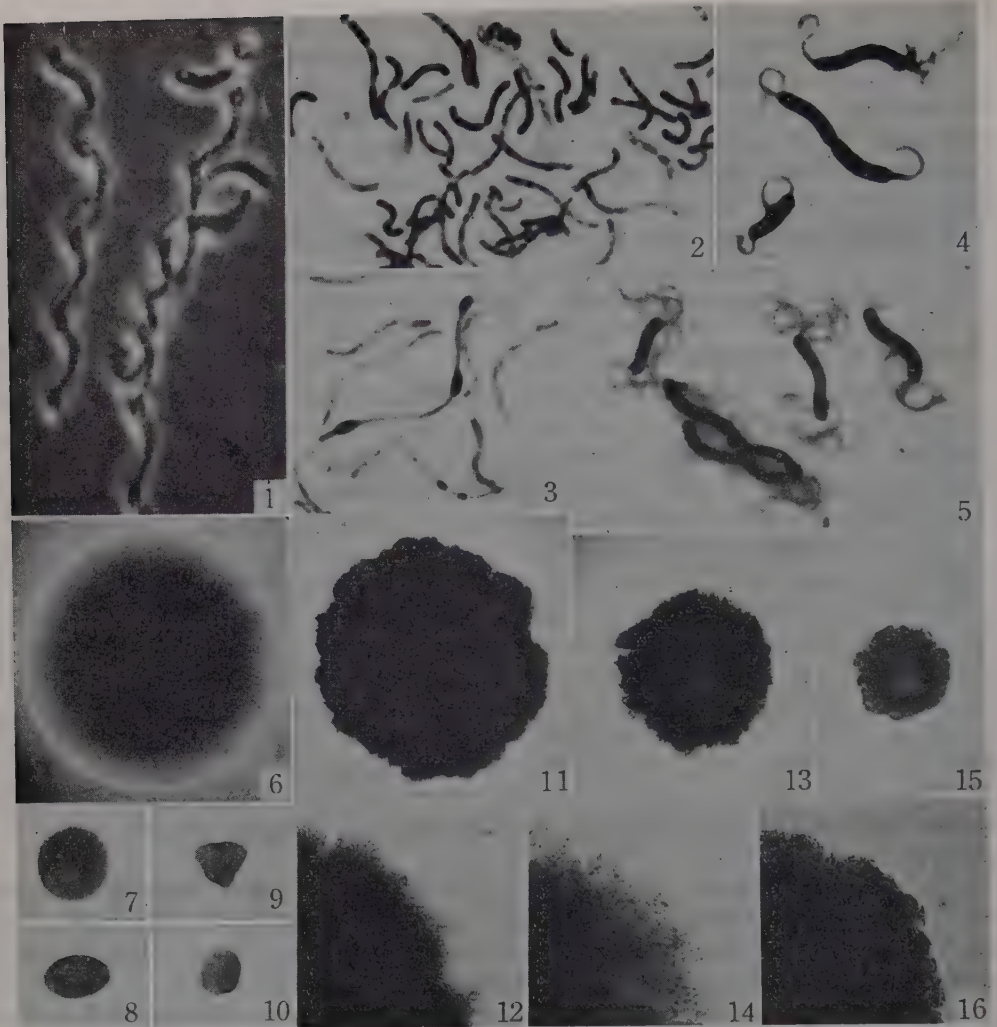
Gelatin stab: Growth is seen along the entire stab, but it is scanty in the lower part of the stab. Growth on the surface is limited at the point of puncture. The line of puncture is villous or beaded, and the gelatin is very slowly liquefied in crateriform. After 5 weeks, liquefaction is about 7 mm. in depth.

Nutrient broth: Growth is speedy. In 24 hrs. at 35°, thin membranous masses develop on the surface, and a thin ring develops along the tube wall. The medium is turbid. The masses are precipitated by slight stimulus. The ring becomes weaker with the culture ages, and disappears in about 5 days. The broth becomes clear in about 2 weeks. Sediments diffuse uniformly by shaking the tube. Fetid odor is absent.

Potato: No growth occurs.

(3) Physiological characteristics

The spirillum grows only in the range about 3 mm. from the surface of the medium in shake culture. It does not reduce nitrate to nitrite, and does not produce indole and hydrogen sulfide. Catalase is not produced by growth on the nutrient agar. The spirillum produces neither acid nor gas from glucose, fructose, sucrose, lactose and mannitol. Litmus milk remains unchanged. The optimum temperature for growth is 35°. The organism can grow at 40°, but not at 44°. The optimum pH for growth is 6.4 to 7.4, and growth can be initiated between pH 5.6 and 9.2. Methyl red and Voges-Proskauer reaction are negative. The spirillum utilizes ammonium salts and asparagine as a sole nitrogen source in synthetic media, but not nitrate and



Spirillum putridiconchylium nov. sp.

Fig. 1. Vegetative cells moving slowly in the nutrient broth after incubating for 24 hrs. at 35°. $\times 1500$ (photographed with a dark contrast phase microscope).

Figs. 2 to 3. Specimens stained with Löffler's methylene blue. $\times 1500$. The cells in Fig. 2 are obtained from a colony grown on the nutrient agar plate after 24 hrs. at 35°. The cells in Fig. 3 after 72 hrs. at 35°, and two spirilla among them contain the volutin granules.

Figs. 4 to 5. Flagella-staining by Löffler's method. $\times 1500$. The cells in Fig. 4 are obtained from a colony grown on the nutrient agar plate after 24 hrs. at 35°. The cells in Fig. 5 after 72 hrs. at 35°.

Fig. 6. A surface colony on the nutrient agar plate after 24 hrs. at 35°. $\times 30$.

Figs. 7 to 10. Deep colonies on the nutrient agar plate after 24 hrs. at 35°. $\times 30$.

Fig. 11. A surface colony on the nutrient gelatin plate after 7 days at 20°. $\times 30$.

Fig. 12. The edge of the same colony as in Fig. 11. $\times 90$.

Fig. 13. A deep colony near the surface of the nutrient gelatin plate after 7 days at 20°. $\times 30$.

Fig. 14. The edge of the same colony as in Fig. 13. $\times 90$.

Fig. 15. A deep colony far from the surface of the nutrient gelatin plate after 7 days at 20°. $\times 30$.

Fig. 16. The edge of the same colony as in Fig. 15. $\times 90$.

urea. The spirillum utilizes the salts of succinic, fumaric, pyruvic, and malic acid in synthetic media, but not the salts of acetic, propionic, butyric, lactic, citric and malonic acid. Also glucose, fructose, glycerol, and ethyl alcohol are not utilized.

Discussion

The spirillum isolated by the present author is catalase-negative. Giesberger³) diagnosed that *Spirillum* is catalase-positive, but thereafter a species of *Sp.*, *Sp. mancuniense* which was catalase-negative was isolated by Cayton *et al.*⁴). Williams *et al.*⁶) have described that “—if other catalase negative spirilla species are isolated, this characteristic will have to be included in the diagnosis of the genus—”. More recently, four species of *Spirillum* which were catalase-negative were isolated by Watanabe⁷). From the above-mentioned reason, the present author concluded that this characteristic has to be employed as a key for identification of *Spirillum*. Although the spirillum is in accord with these species in the point that it is catalase-negative, it is markedly different from them in several other important respects. The spirillum in question has some resemblance to the following species: *Sp. undula*, *Sp. sinuosum*, *Sp. graniferum*, *Sp. virginianum*, *Sp. serpens*, and *Sp. serpens* var. *azotum*. Therefore discussion must be put on their morphological, cultural and physiological properties. All the species except *Sp. virginianum* have almost the same size in diameter of the cell though they differ more or less in the number of spirals. The spirillum in question, therefore, cannot be distinguished from the others in its morphological properties. But in the cultural and physiological properties, the spirillum has some points of dissimilarity to each of them. It can be concluded that the spirillum in question is another species from *Sp. sinuosum* and *Sp. graniferum* because it differs from the others in the ability to liquefy gelatin, in the catalase-reaction, in the sort of the nitrogen and carbon compounds that can be used for growth in the synthetic media, and in several other less important points. Although *Sp. undula* liquefies gelatin, it differs from the spirillum in question in the color of the colonies on gelatin, in the catalase-reaction, in the sort of the carbon compounds, in the optimum temperature for growth, and in several other less important points. *Sp. virginianum* fairly resembles the spirillum in question in the point that it has been isolated from a shell fish, and in several other physiological properties. But also *Sp. virginianum* is a different species from the spirillum in question because the former differs markedly from the latter in the ability to liquefy gelatin, in the catalase-reaction, in the sort of carbon compounds, and in several other less important points. It is *Sp. serpens* that has many points of similarity to the spirillum in question. However, *Sp. serpens* (the descriptions of *Sp. serpens* in Migula's Manual⁸), Giesberger's Monograph, and Bergey's Manual differ from one another in some points.) has some points of dissimilarity to the spirillum in question. The points of difference between *Sp. serpens* in each of investigators and the spirillum isolated by the present author are expressed in Table 1.

As shown in Table 1, the spirillum isolated by the present author differs from *Spirillum serpens* in some important respects for identification even if it is compared with any one of *Spirillum serpens* by these investigators. Also *Spirillum serpens* var. *azotum* differs from the spirillum in question in the inability to liquefy gelatin in addition to those characteristics.

From the above points of view, the spirillum isolated by the present author is diagnosed as a new species, and is named *Spirillum putridiconchylum*.

Table 1. The points of difference between *Spirillum serpens* and the new *Spirillum*.

	<i>Spirillum serpens</i>			The spirillum isolated by the present author
	in Migula's Manual	in Giesberger's Monograph	in Bergey's Manual	
Agar colony	surface: colorless in edge, yellow in center deep: yellow to brown; whetstone form	surface: cream; undulate	surface: heavy cream color	surface: creamy-white; entire deep: creamy-white; round, elliptical, or spindle
Gelatin colony	surface: colorless in edge, light, yellow in center; lobate deep: greenish yellow to brown; round		surface: yellow to brownish; entire	surface: brownish-white; undulate or lobate deep: brownish-white; lacerate, or undulate
Potato	white; thick	clear orange yellow	clear orange-yellow	no growth
Catalase		positive	positive	negative
Available carbon compound		salts of acetic, butyric, succinic, lactic and pyruvic acid		salts of succinic, malic, pyruvic and fumaric acid
Available nitrogen compound		ammonium salts		ammonium salts and asparagine

The author is grateful to Dr. Teijiro Kishitani, the President of Suzugamine Women's College, for his valuable advice and criticism during the course of the study. Thanks are also due to Mr. Narumi Watanabe, Assoc. Prof. of Faculty of Education, Chiba University and to the late Mr. Masatoshi Kosaka, the Chief of Medical Branch of the Library, Hiroshima University, for their kindness in collecting literatures which has been cited.

Summary

A new species of *Spirillum* was isolated from a fresh water snail, *Semisulcospira bensoni* (Philippi) which had been putrefied in a Petri dish in our laboratory. From the morphological, cultural and physiological points of view, it is diagnosed as a new species and named *Spirillum putridiconchylum*.

References

- 1) Ehrenberg, C. G., Physik. Abhandl. K. Akad. Wissensch. Berlin, p. 38 (1832).
- 2) —, Die Infusionsthierchen als vielkommende Organismen. Leipzig, (1838).
- 3) Giesberger, G., Beiträge zur Kenntnis der Gattung *Spirillum* Ehrbg. Delft, (1936).
- 4) Cayton, H. R., and Preston, N. W., Jour. gen. Microbiol. **12**: 519 (1955).
- 5) Bergey's Manual of Determinative Bacteriology 7th ed. (1957).
- 6) Williams, H. A., and Rittenberg, S. C., International Bull. of Bacteriological Nomenclature and Taxonomy **7**: 49 (1957).
- 7) Watanabe, N., Bot. Mag. Tokyo **72**: 77 (1959).
- 8) Terasaki Y., Bull. of Suzugamine Women's College **5**: 7 (1958).
- 9) Migula, System der Bakterien. Bd. II: 1022 (1900).

摘 要

寺崎 弥 助: 新種 *Spirillum putridiconchylum* について

1958 年 8 月, 広島市古田町で採集され, 実験室において腐敗した淡水産巻貝, カワニナに, 多数の螺旋形微生物が生育していた。この腐敗液を, ペプトン, 酵母エキス, カワニナせんじ汁, 食塩を含んだ寒天平板に塗抹することにより, 容易に *Spirillum* を分離しえた。分離された *Spirillum* は約 2 年間試験管内で植えつがれた現在も当初と同様に, 明瞭な左巻きの螺旋形を示す。カタラーゼ陰性で, 無機窒素源としてアンモニウム塩は利用できるが, 硝酸塩は利用できない。本菌を培養し, 形態学的, 生理学的性質をしらべ, 正確な種として記載されている *Spirillum* と比較検討した結果, 新種と断定したので, 腐敗した貝より分離されたことにより, *Spirillum putridiconchylum* と命名した。(鈴峰女子短期大学生物学教室)

書 評

生物の進化、進化論の解答と問題

Heberer, G.: Die Evolution der Organismen. Ergebnisse und Probleme der Abstammungslehre. 2. erweiterte Auflage, Gustav Fischer, Stuttgart. 1~3 (1954), 4 (1955), 5, 6 (1957).

ドイツにおける専門家を網羅して集大成された進化生物学の専門書であり、「基礎と方法」「生物の歴史」「系統の因果性」「人間の系統」の6分冊で、1,000ページをはるかに越す大部のものである。

これらの内容を分担者によって示すと次のとおりである。第1分冊「基礎と方法」172ページ, Dingler, H. 「進化論の哲学的基礎」, Zimmerman, W. 「系統学の方法」, Rensch, B. 「個体発生の系統的变化」, Lorenz, K. 「心理学と種族の歴史」. 第2分冊「生物の歴史」175~422ページ, Rüger, L. 「系統の時間的尺度としての地質時代の絶対時間」, Weigelt, J. 「種族の歴史の証拠研究としての古生物学」, Friedrich-Freska, H. 「ウィールスの種族史的位置と偶然発生の問題」, Mägdefrau, K. 「植物の歴史」, Remane, A. 「動物の歴史」. 第3分冊 425~712ページ「系統の因果性」で, Schwanitz, F. 「植物の遺伝と進化の研究」, Lüers, H. und Ulrich, H. 「動物の遺伝と進化の研究」, Ludwig, W. 「選択説」. 第4分冊 713~856ページ「系統の因果性」の続き, Schwanitz, F. 「全植物界の進化のモデルとしての有用植物の起原」, Herre, W. 「馴養と種族の歴史」. 第5分冊前半 857~914ページは「系統の因果性」の続きで, Heberer, G. 「加重的型発生説」. 後半917~1109ページ「人間の系統」, v. Krogh, G. 「霊長類における人間の位置」, Gieseler, W. 「人類化石の歴史」. 第6分冊は「人間の系統」の続きで, Reche, O. und Lehmann, W. 「人間における人種形成の遺伝」, v. Eckstedt, E. 「精神の全歴史(化石心理学)」, 索引となっている。各項目には文献がつけられているので, 植物学, 動物学, 人類学, 古生物学のあらゆる分野の人, 分類学, 細胞学, 形態学, 発生学, 遺伝学などの専門家も必ず興味ある項目があるのだから, 図書館に是非とも備えつけたい本である。

特に植物に関する部分について, もっと内容をくわしく紹介したい。「植物の歴史」では, シダ植物がプロフィトン類から分かれたのであろうが, 小

葉シダと大葉シダとに分けられるというか, 小葉シダの仲間トクサ, 楔葉木, ヒカゲノカズラ, クラマゴケ, 封印木, 鱗木などから裸子植物のコルダイテス, イチョウ, 松柏類が系統的に近縁になっていて, 被子植物の一部まで続く。大葉シダのシダ類に近いシダ種子植物からベネチテス, ソテツ, *Caytonia* が出て, この *Caytonia* から被子植物のウマノアシガタ目が生じたと考えられている。このように維管束植物のシダ類, 裸子植物, 被子植物は系統的な単位ではなく, 体制の段階を示すもので, Psilophytinae→Lycopodiinae→Coniferales (→? Angiospermae) または Psilophytinae→Filicinae eusporangiatea→Pteridospermae→Cycadales (および Pteridospermae→Caytoniaceae→Angiospermae p. p.) の系統が考えられている。

「植物の遺伝と進化の研究」では, 形態多様性の原因として, (1) 遺伝子の突然変異性 (2) 染色体突然変異 (構造変化でオオマツヨイグサなどの染色体環形成) (3) ゲノム突然変異 (異数性, *Datura* の三染色体植物, 倍数性) の多数の例があげてある。形態制限の原因として, (1) 選択 (2) 隔離の例があげられ, *Layia glandulosa* の種内交雑, *Holocarpa* 属の4種間交雑, *Layia* 属や *Viola* 属の交雑の難易が示されている。最後に遺伝学と種概念, より大きい分類学上の単位および遺伝学と不可逆性の法則で稿をとじている。染色体やゲノム突然変異の不可逆性のはっきりした原因をつかめば道を誤まることはなからうと。

「進化のモデルとしての有用植物の起原」では野生種から有用植物の由来で栽培植物の巨大型, 野生種と栽培種の他の差異, 栽培植物のいろいろの器官の変化, 平行変異などが注目される。栽培植物の起原に対する遺伝学的基礎としては, 遺伝子突然変異, 交雑による優良遺伝子の組み合わせ, 染色体突然変異, ゲノム突然変異 (異数性と倍数性, 人為倍数体の誘導), 植物育種に対する細胞質遺伝の重要性があげられる。環境の意義に対しては, 環境要因と生じる突然変異の効果, 一次および二次作物, 有用植物の発生地, 自然および人為選択が考えられる。最後に人間の進化過程に結びついた植物の育種が考えられ, 植物育種の目標と可能性が説かれている。

(佐藤重平)

抄

録

海産植物の光合成と塩分濃度

Gessner, F., und Hammer, L.: Die Photosynthese von Meerespflanzen in ihrer Beziehung zum Salzgehalt. *Planta* 55: 306-312 (1960).

ヒルムシロ科の海産植物 *Posidonia oceanica* の葉および緑藻 *Ulva lactuca* の葉状体を用いて、最初海水中で 20 分間、その後淡水中に移して 20 分間、水温 22.2°C で 15000 lux の光をあてて光合成を測定した。植物を海水から淡水に移すと、みかけの光合成は *Posidonia* では 0 またはマイナスの値にまでさがり、*Ulva* では海水中で示した値の約 23% まで低下した。淡水中から再び海水中に戻すと光合成は再び急激に高まるが、最初の海水中における値にまで完全には回復しなかった。しかし、20 分間隔で両植物を海水から淡水へ、淡水から海水へと移しかえると、ちょうど光源の点滅に応じて植物が光合成を行なったり停止したりするのと同じく同じ正確さで、光合成が低下したり回復したりする。この変動を 20 分間隔で 1.5~2.5 時間にわたって数回くりかえすことができた。種々の濃度にうすめた海水中で *Posidonia* の光合成を測定した結果、濃度 100~40% までは濃度の低下とともに光合成は海水中の値の約 80% まで徐々に低下し、それ以下では濃度 20% のとき光合成は約 40%、濃度 0% (淡水) では光合成 0 と急速な低下を示した。それぞれの塩分濃度に対応した光合成の値が存在するようにみえるが、このことは光合成系とそれを取りまく medium の間に平衡状態が存在することを示唆する。Medium の変化に対して植物が非常に急速に反応することから、植物を海水から淡水に移したり、淡水から海水に戻したりするときの光合成速度の変化には、細胞内の状態の変化ではなくて、膜の状態の変化が関係しており、これが CO₂ 透過の量を規制し、従って光合成の限定要因になっていると推定される。(有賀祐勝)

カサノリにおける細胞質リボ核酸の

自主合成に関する研究

Naora, H., Naora, H., and Brachet, J.: Stu-

dies on independent synthesis of cytoplasmic ribonucleic acids in *Acetabularia mediterranea*. *J. Gen. Physiol.* 43: 1083-1102 (1960).

核-細胞質相関性を追求するため単細胞のカサノリを無核片と有核片とに分けて比較する試みにはすでにいくつかの報告があるが、ここでは除核後の RNA の消長を検討した。細胞質 RNA の大部分約 81% は葉緑体に局在する。除核後も全 RNA 量には著しい変動はないが、葉緑体 RNA はなおいくぶん増加し、ミクロゾームや上澄部分(M・S 部と仮称)では急速に低減する。有核部では葉緑体 RNA 量は 20 日後には初めの 140% にまで著しく増加するが M・S 部でも増加している。Guanine の添加は両片ともに害に働き、8-azaguanine は有核では無影響、無核ではかえって対照の 16% も促進する。¹⁴C-adenine は主として葉緑体 RNA にとりこまれ M・S 部には少ないが、さらに無核では葉緑体にのみ認められ、M・S 部では放射活性は検出されない。¹⁴C-orotic acid も細胞質 RNA にとりこまれるが有核部の方がより急速で 10 日後 有核/無核=1.33 であった。¹⁴CO₂ も同じく RNA に入るが有核部では 20 日後に無核部の 2 倍の価を示した。これらの結果は葉緑体が細胞質における RNA 合成の最も主要な場であって、しかも核なしでも RNA の新規合成をなしうるが、M・S 部は核の存在においてのみその合成と維持がなされていることを明らかにしている。しかし核を除いても ¹⁴CO₂ は酸可溶プリン中にはとりこまれるので、この第一段階はあまり核制御をうけず、次にそれが RNA にとりこまれる第二段階が間接的であるが、核の遠隔制御をうけるらしい。要するに葉緑体は核の仲介なしに自律的に RNA 合成の能力をもつものである。(……と著者らは特に強調しているが、有核と無核とを比較するとき、その差はきわめて大きいのであって、この事実を軽視しているのはまだ論議が残されている感じがする。……訳者註) (吉田吉男)

本 会 記 事

支 部 通 信

北海道支部

2月例会（昭和36年2月25日，北大理学部において）

豊国秀夫：ミヤマアケボノソウの変異性と分類上の位置について，酒井昭：植物の凍害に対する諸物質の保護作用とその関連性

関東支部

2月例会（昭和36年2月25日，東大理学部において）

竹村英一：ヒガンバナ属の人工雑種とその系統，相見霊三：イネの種実における澱粉の蓄積機構に関する細胞生理学的研究

中部支部

1月例会（昭和36年1月28日，愛知県立女子大において，日本動物学会中部支部と共催）

「高中学校の生物学教育の現状と将来」についての懇談会

北陸支部

第10回総会および第39回例会（昭和35年5月15日，丸岡高校において）

井原正昭：ネギ属の分類学的研究，香室昭門：西南日本の溜池をたずねて，柴田万年・堺恵美：チューリップの品種から単離されたアントシアニンについて，里見信生：御蔵島旅行談

第40回例会

エクスカーション

第41回例会（昭和35年10月8日，金沢大理学部において）

正宗敬敏・里見信生：ヤマモモの園芸品種の分類学的知見，西田晃二郎：光合成の初期産物としてあらわれるマルトースについて

第42回例会（昭和35年12月10日，富山市産業教育会館において）

大島哲夫：ヒマワリの根の一部切除による生長の変化について，井原正昭：外国産ネギ属の二，三種について，本多啓七：日本北アルプスにおける隠花植物の生態について

第43回例会（昭和36年2月11日，金沢大理学部において）

瀬嵐哲夫：コウジカビのアミラーゼに関する遺伝生化学的研究，柴田万年・石倉成行：チューリップの一品種 Charles Needham のアントシアニン

昭和36年度北陸支部長は正宗敬敏氏に決まりました。

会 員 住 所 変 更

昭和35年8月—昭和36年2月
括弧内は自宅，支部別到着順

東北支部

倉石 衍 東北大農芸化学
奥田 慎一

関東支部

新井 養 老 (文京区白山前町1)
仙波 安 芸 中央区銀座東7丁目 オリエンタル・ブックス
橋本 徹 武蔵大生物(大田区女塚2の9)
津村 孝 平 (横浜市神奈川区松ヶ丘14)
木下 三 郎 (目黒区上目黒8の683大橋住宅RF-36)
鈴木 静 夫 東京理科大薬学微生物化学
稲葉 二 郎 (埼玉県草加市両新田高橋住宅RF-36)
入来 義 彦 信州大教育生物
清水 建 美 信州大繊維生物
釜野 井 正 雄 世田谷区玉川用賀町3-7 東京農大育種学研
荒井 清 司 (長野県中野市松川県住宅10号)

中部支部

鈴木 米 三 (神奈川県伊勢原町子易759)
楠 正 貫 (名古屋市千種区虹ヶ丘南住宅10-304)
山本 幸 男 名大理生物
高橋 久 之 (藤枝市益津16-3)
桜井 昌 (浜松市名残町本町)(旧姓百瀬)

近畿支部

稲葉 通 一 (姫路市大津区天満1115 三輪方)
須之部 淑 男 (大阪府枚方市中宮第2団地16号の106)
深瀬 嶽 和歌山市西浜1596の1 和歌山市立和歌山商業高校

池田勝彦 堺市大仙町大阪府立大農遺伝育種研
三井早苗 (大阪市阿倍野区相生通 1-65)
岸本卯一郎 (川西市寺畑釜割谷 3-9)
田村道夫 大阪府豊中市柴原 阪大教養生物 (大阪府吹田市千里山公園住宅虹ヶ丘 2-302)

中国・四国支部

大島貞男 鳥取県米子市 県立米子南高校
益森静生 山口市芳沢町 山口大教育生物

九州支部

大橋裕 長崎大薬学生薬
藤木素士 (熊本市京町本丁69 佐伯方)

新入会員

北海道支部

猪田俊彦 網走市北10条西4丁目網走市立第二中学 (同市北2条西2丁目鈴木五郎方)
松川浩彬 北海道学芸大函館分校生物 (函館市杉並町 200 小田桐方)
笠原和男 北大理海藻研
岩淵雅樹 北大理植物
奥出功 北海道上川郡東川町立東川中学 (上川郡東川町西4号北4)
中条良作 北海道上川郡上川町 層雲峡博物館
西田達郎 北海道網走郡美幌町字報徳 美幌高校 (北海道網走郡美幌町西2条南3丁目)

東北支部

山本光男 山形大文理
沼辺征一郎 宮城県気仙沼市字町裏66 県ヶ浦高校
佐々木準次 秋田県鹿角郡十和田町大湯 秋田県立十和田高校大湯分校
五十嵐彰 福島県伊勢郡梁川町 県立梁川高校
栗田精一 宮城県名取郡岩沼町字桜47 宮城県立名取高校 (仙台市長町字門前町 13-1)

関東支部

渡辺まつ枝 千葉市小仲台町 824 千葉第二高校 (千葉市小仲台町 1743-1)
右手和夫 東京都大島町差木地 都立大島高校差木地分校
吉野昭朗 千葉県東葛飾郡我孫子町 電力中央研究所農電研
石井隆文 東京教育大理植 (川崎市大島町 3-60)

志村英二 平塚市中原 1519 農技研 (平塚市中原 1519 農技研内)

南沢吉三郎 小金井市中町 2丁目 農工大繊維

蛭原富男 水戸市愛宕町 2182 茨城県蚕業試験場

勅使河原司郎 群馬県前橋市緑社町 群馬県蚕業試験場

杉村康知 千葉県習志野市大久保 東邦大理生物

三井清司 東京教育大理植

斎藤規夫 東京教育大理植

新井澄 長野県諏訪郡原村 原中学 (長野県諏訪郡原村松沢 鎌倉充男方)

石井稔 千代田区神田猿楽町 2-4 韓国YMCA内 韓星貿易商会

金井竜二 東大理生化

金井康彦 大田区南千束町46 学習研究社 (杉並区大宮前 6-340)

桜井英博 東大理生化

徳田広 東大農水産植物 (横浜市中区西之谷 71)

稲本賢次郎 千代田区神田猿楽町 2-4 韓国YMCA内 鶏林書房

山崎弥三郎 茨城県東海村 日本原子力研究所 (東京都豊島区巣鴨 1-100)

岡田稔 目黒区上目黒8-500 津村研究所 (杉並区松ノ木町 1144)

福田泰二 東大理植

杉山有 川崎市久本180 川崎市立高津高校 (江東区北砂町 6-36)

長秀彦 (栃木県鹿沼市久保町 1609)

四分一平内 埼玉県秩父市大宮1696 県立秩父高校 (埼玉県熊谷市柿沼 5)

村松秋清 (甲府市川田町 517)

中部支部

杉浦昌弘 名大理生物 (名古屋市中千種区唐山町 174 加藤錠一方)

藤田達也 静岡県掛川市掛川1225 県立掛川西高校 (掛川市瓦町)

栗田子郎 静岡県小笠郡浜岡町 県立池新田高校 (小笠郡菊川町堀之内 244)

近畿支部

片山忠夫 京大農応用植物

川戸峯子 大阪市東住吉区平野流町 大阪学芸大平野分校生物 (神戸市東灘区本山町森神岡)

西門義一 高槻市常盤町66 西門南類研 (大阪府大槻市常盤町 66)

- 中 村 公 弘 大阪市此花区春日出町 364 大
阪市立梅香中学
柳 進 (京都市東山区山科音羽稻芝町
35-3 栗津方)
丸 重 啓 二 京大農応用植物
丸 山 圭 蔵 京大理植
木 村 和 義 京大農応用植物
平 岩 公 男 天理市仙之内町 天理大 (天理
市若木町 12)
仲 井 啓 郎 兵庫県水上郡柏原 県立柏原高
校生物
森 下 日 出 旗 大阪市立大理
舟 根 昇 京都市東山区山科大宅坂の辻町
日本新薬株式会社山科薬用植物
研
沖 敏 行 大阪大理纖維研
奥 田 正 男 大阪市立大理植

中国・四国支部

- 三 原 勉 松山市東長戸 640 松山市立鴨
川中学 (松山市西須賀町 2245-
2)
三 好 茂 正 松江市西川津町 島根大生物 (島
根県八束郡八雲村東岩坂 249)
勝 本 謙 山口大農 (下関市長府町中の町)
友 永 進 (山口県吉敷郡大内町下矢田 中
原福一方)
中 島 秀 明 広島大理植
築 地 正 勝 " "
寺 内 啓 作 " "
吉 川 和 之 " "
岡 本 修 治 " "
安 達 貞 夫 " "
田 羅 征 伸 " "
生 塩 正 義 " "
三 好 教 夫 " "
橋 本 清 美 " "
小 野 完 爾 " "

- 芝 元 和 朗 広島大理植
遠 藤 信 雄 " "
岡 嘉 昭 " "
岡 畑 寛 " "
松 田 忠 男 " "
浅 田 弘 治 " "
山 下 博 太 郎 広島県佐伯郡五日市町 明治製
菓広島工場
橋 本 静 子 広島大理植 (広島市東雲町 574
-3)
友 田 行 雄 山口県能毛郡上関町 能毛南高
校上関分校 (山口県玖珂郡由宇
町中倉)

九州支部

- 上 田 英 次 大分県宇佐郡院内村大副 東院
内小学校 (大分県宇佐郡駅川町
樋田)
坂 口 信 治 鹿児島大文理生物 (鹿児島県川
内市川内駅 駅長宿舎)
秋 武 和 俊 九大理生物
遠 藤 正 喜 大分市碩田中学 (大分市北新町
6 組)

外 国

- 張 鳳 烈 (韓国釜山市西区富民洞一街36)
河 永 亡 韓国京城市城北区安岩洞
高麗大学校生物
洪 淳 佑 " "
李 德 鳳 " "
丁 年 碁 韓国釜山市東萊区 金井国民学
校
鄭 英 昊 韓国京城市 京城大学校文理科
大植
鄭 佐 秀 韓国慶南晋州市 晋州農科大図
書館

S. Sampath Cytologist, Central Rice Re-
search Institute, Cuttack 4,
Orissa, India.

Studies of the Germination of the Spore in Some Mosses II. *Diphyscium fulvifolium* Mitt. and *Sphagnum* *cuspidatum* Ehrh.

By Yuko NISHIDA* and Shintaro SAITO**

Received April 23, 1960

The general mode of the development of the protonema in several species of *Diphyscium* and *Sphagnum* was reported by some authors (Campbell¹), Müller²), Noguchi³), Noguchi and Muraoka⁴)), but there has not appeared any description of development of protonema in sporelings of *Diphyscium fulvifolium* Mitt. and *Sphagnum cuspidatum* Ehrh. This time the present writers will report the results of the observations on spore-germination of these mosses. In addition, influences of four different methods of culture on the form of the protonema of *Sphagnum cuspidatum* will be described.

Materials and Methods

The collected sporogonia were wrapped up in paraffine paper and had been kept dry until the cultures were undertaken. All the cultures were placed near a window, to be illuminated by diffused daylight, any direct rays of the sun being avoided. The cultures were kept at room temperature.

Spores of *Diphyscium fulvifolium* were sown on a porous plate (7×7×1 cm.) which was placed in a sterilized Petri-dish filled with Benecke's solution (pH, 5.5-6.0), keeping its upper surface above the surface of the solution. The cultures started on November 5, 1957 and finished late December of the following year.

Spores of *Sphagnum cuspidatum* were sown (1) in Benecke's solution, (2) on 1% Benecke's agar, (3) on sterilized filter paper moistened with Benecke's solution, and (4) on sterilized porous plate moistened with the same nutrient solution. The pH value of all these culture media was 3.5-4.0. The cultures began on April 14, 1959, and continued until late September of the same year.

Results

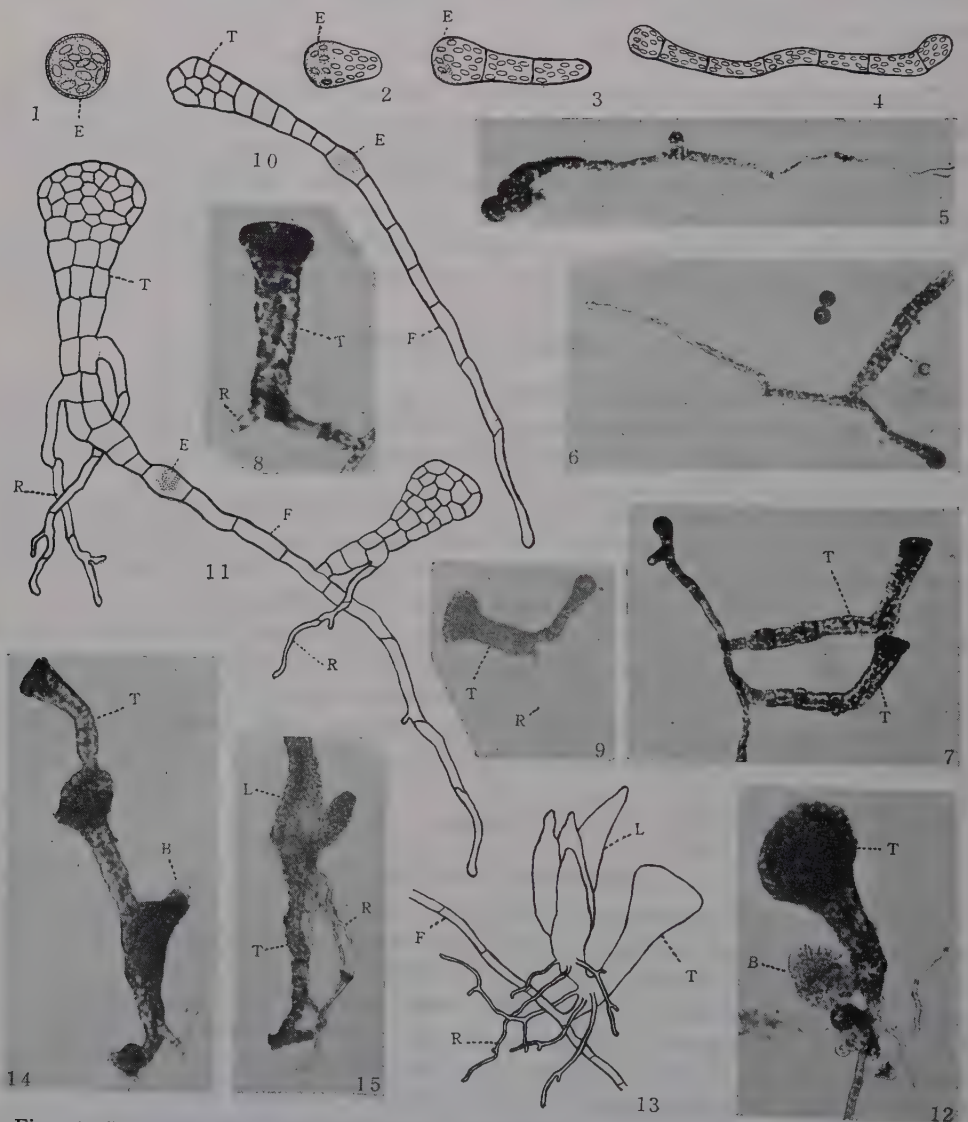
Diphyscium fulvifolium Mitt.

The spores of this species were collected at Atagoyama, a low hill in Matsue City, Shimane Prefecture on August 13, 1957. The spores of this species are about 17-20 μ in diameter and swell up to about 25-30 μ within 2 or 3 days of the culture. The number of the chloroplasts in the spore increases gradually until they germinate. At the end of the fourth week of the treatment, a germ tube is formed as a protuberance through the rupture of the exospore. No further development of the germ tube was observed during the winter until early April of the following year, when the germ tube regained activity. It develops into a filamentous protonema by successive cell divisions. The completed filamentous protonema is only 450-850 μ in length with a few short branches, and tapers gradually towards one end turning into a terminal rhizoidal filament. The cells of the rhizoid bear oblique cross walls and contain a

* Middle School, Faculty of Education, Shimane University, Shimane, Japan.

** Institute of Biology, Faculty of Literature and Science, Shimane University, Shimane, Japan.

few chloroplasts, or frequently lack them entirely. In some cases the endospores produce two chloronemal filaments in two opposite directions, each bearing a terminal rhizoid (Fig. 17 C), or, in some other cases, they produce a rhizoidal filament in one direction and a chloronemal filament in the other (Fig. 17 B). In early June, 1 to 3 spherical protuberances appear on the chloronemal cells of the green filament. The protuberance becomes larger rapidly, and is partitioned by a transverse septum from



Figs. 1-15. *Diphyscium fulvifolium* Mitt. 1-3. Formation of the filamentous protonema, (1, 2, 3, $\times 600$). 4-11. Formation of the trumpet-shaped protonema, (4, $\times 400$; 5-11, $\times 350$). 12, 13. Formation of the leafy plant, (12, 13, $\times 380$). 14. The first, the second and the third trumpet-shaped protonemata and a leafy plant, ($\times 280$). 15. A leafy plant which arose on the apical part of the trumpet-shaped protonema, ($\times 280$). E: exospore, R: rhizoid, T: trumpet-shaped protonema, F: filamentous protonema, B: bud of leafy plant, L: leafy plant, C: clavate branch.

the main filament, thus becoming a huge spherical initial cell of the clavate branch (Figs. 5-7). The first division wall in the initial cell is always transverse, dividing the initial cell into a basal cell and an apical cell. The basal cell does not undergo any further cell divisions, while transverse divisions occur in the apical cell repeatedly, to form a clavate branch consisting of 8-15 cells (Fig. 6).

The cells of the clavate branch, the basal cell excepted, are divided into quadrants by two longitudinal walls perpendicular with each other (Fig. 16 A); the cells above the level b-b of Fig. 16 continue further cell divisions in various directions. As a result of such cell divisions, the clavate branch develops into a trumpet-shaped protonema (Figs. 7-11). The mature trumpet-shaped protonema contains numerous chloroplasts, and its upper surface is slightly concave (Fig. 16 T). This protonema rises upwards from the procumbent filamentous protonema showing positive phototropic character. In late June, from the basal part of the trumpet-shaped protonema arose several rhizoids provided with a few short branches. These rhizoids bear oblique septa as the terminal rhizoid (Figs. 8, 9, 11, and 12). The terminal cell of the filamentous protonema can very often produce a trumpet-shaped protonema in the same way as mentioned above for the lateral ones (Figs. 9-11, 14, 17 D, E, F). Thus, six types can be distinguished in the formation of the protonema system as shown diagrammatically in Fig. 17 A-F.

The leafy bud formation began in early September. A large elliptical protuberance arises from a certain cell near a rhizoid of the tubular part (a-a or b-b level of Fig. 16) or from a cell of the upper margin of the trumpet-shaped protonema (Figs. 12-15). This protuberance contains many chloroplasts and becomes spherical before it undergoes cell divisions. The first division is oblique. The basal cell thus formed does not undergo any further divisions like as the basal cell of the clavate branch. Two successive oblique divisions in the upper cell produce a pyramidal apical cell with three cutting faces parallel to its side walls. This apical cell and cells beneath it continue further cell divisions forming a globose cell mass, from which, in the mean time, a juvenile stem 4-5 cell long and provided with several juvenile leaves develops (Fig. 12 B). After the differentiation of the juvenile leaves, rhizoids are produced also from the basal part of the juvenile stem (Fig. 13). Usually, only a single leafy plant is formed on a trumpet-shaped protonema.

It is interesting to note that a terminal trumpet-shaped protonema can very often produce another trumpet-shaped protonema from an epidermal cell of its upper surface; a chain of up to three of such protonema is formed in this way. In this case the leafy bud is formed only on the proximal trumpet-shaped protonema during the period of the present cultures.

The development of the sporeling of *D. fulvifolium* is similar to that of the regeneration of the same species reported by Noguchi and Muraoka⁴). However, the mode of the leafy plant-formation in the sporeling and the regeneration of leafy plants differs in that in the latter the leafy plant is derived directly from a cell of the filamentous protonema without any agency of trumpet-shaped protonema, or it is

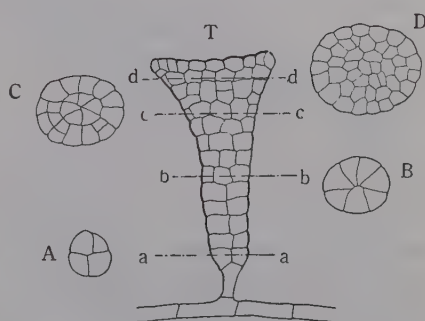


Fig. 16. Cross section of trumpet-shaped protonema. T: trumpet-shaped protonema, A: Cross section at a-a level, B: Cross section at b-b level, C: Cross section at c-c level, D: Cross section at d-d level.

produced on the tip of clavate branches, while in the former it arises always from trumpet-shaped protonema. Müller²⁾ adopted Berggren's figure which indicate that in *D. foliosum* the leafy plants arose from the base of trumpet-shaped protonema.

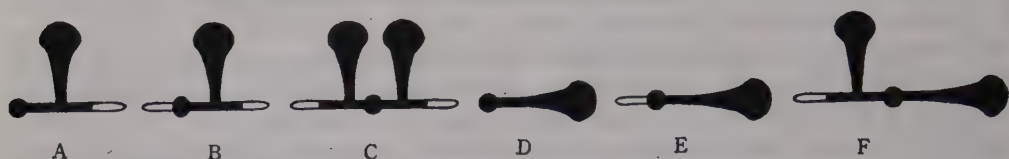


Fig. 17. Diagrams showing six types of protonema system in *Diphyscium fulvifolium* Mitt.

Sphagnum cuspidatum Ehrh.

The spores used in this study were collected from ponds at Uchiama, Higashiizumocho, Yatsuka-gun, Shimane Prefecture on April 9, 1959. The spores are nearly triangularly pyramidal (Fig. 18), about $30\text{--}35\mu$ in diameter, and brown as a whole. They were sown five days after the collection. On the third or fourth day of the culture chloroplasts in the spore much increase in number. In all cultures the spores germinate within about 7–10 days, when the spores are elongated nearly two times as large as the first measurement. A germ tube appears as a protuberance through the rupture in the exospore at a vertex (Fig. 19). The exospore frequently remains on the filamentous protonema until the juvenile leafy plant appears on the thallose protonema. In the cultures (2), (3), and (4) the germ tube develops into a short and chlorophyllose and filamentous protonema, usually consisting of only 2–3 cells, while in the culture (1) the chlorophyllose and filamentous protonema becomes very long (Figs. 29, 30), which is either submerged in the nutrient solution or floated on its surface. The cells of the filamentous protonema which is stained only after a considerable time by the same stain, are stained rapidly by Yanus green B with their chloroplasts except the terminal cell. The terminal cell of the filament becomes larger rapidly (Figs. 20, 21); then it divides into two by an oblique wall, the upper cell thus formed functions as the apical cell of the thallose protonema, and is divided again obliquely by a wall perpendicular to the first. Such a process is repeated several times forming two alternate rows of several cells, which, thereafter, undergo succeeding divisions in various planes resulting in a green, multicellular, one-cell layered thallose protonema (Figs. 22–32). In the cultures (2), (3), and (4), the protonema seems to be completed after a period of about two months, while in the culture (1) the protonema grows more rapidly and is completed after about two weeks earlier than those in the other cultures. In the culture (4), the completed thallose protonema is ribbon-shaped with an irregular outline and is about 2–3 mm. in length (Fig. 31). On the other hand in the cultures (1), (2), and (3), the thallose protonema is palmately lobed. In all cultures the thallose protonema shows the phototropic character.

Rhizoids are formed on filamentous protonema at early stages (Fig. 22). When the terminal cell of the filamentous protonema becomes large, a protuberance appears from cells of the filament; it develops into rhizoids consisting of a few elongated colorless cells (Figs. 25–28, 31, and 32). As the growth of the thallose protonema proceeds, many rhizoids are produced from the peripheral cells of its basal region (Fig. 25), while the upper part of the thallose protonema is free from rhizoids (Figs. 25, 26, and 31). In this moss, a rhizoid frequently arises directly from the endospore as is shown in Fig. 23. On the mature protonema about 8 to 15 rhizoids are born (Figs. 27, and 31). The protonema formation is similar to that of *Sph. squarrosum* reported

by Noguchi³). He reported also the formation of the second, and the third (and the fourth in rare cases) protonema for *Sph. girgensohnii*, but such a phenomenon was

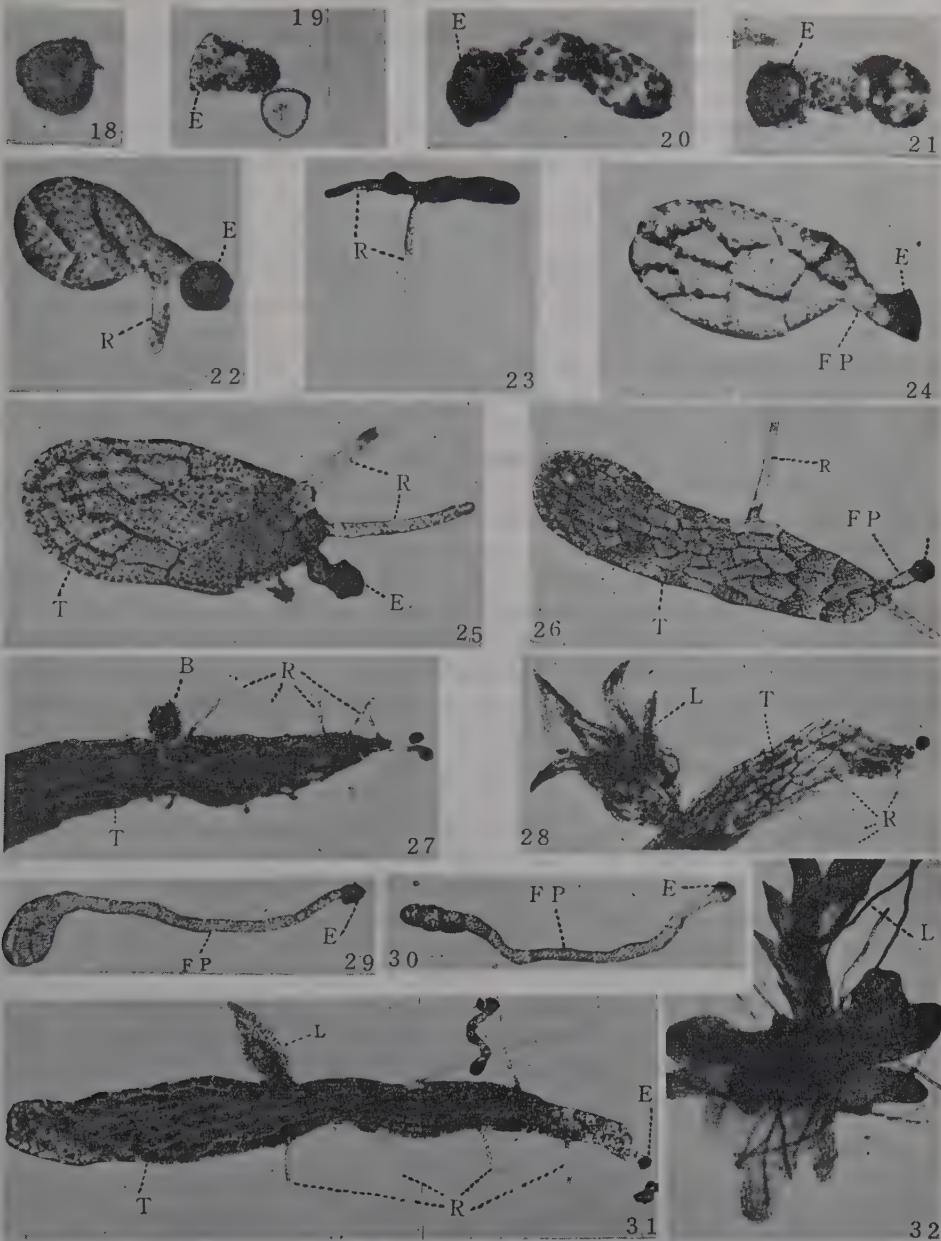


Fig. 18-32. *Sphagnum cuspidatum* Ehrh. 18. Spore, ($\times 480$). 19. A germ tube and dead spore, ($\times 400$). 20,21. Filamentous protonema patterns, ($\times 420$). 22-26. Formation of thallose protonema, (22, $\times 400$, 23, $\times 150$, 24, $\times 360$, 25, $\times 240$, 26, $\times 160$). 27,28,31. Formation of leafy plant, ($\times 100$). 32. Palmate-shaped thallose protonema on agar medium, ($\times 80$). 29. Long filamentous protonema grew on a very damp medium, ($\times 140$), 30. Side view of ditto, ($\times 140$). T: thallose protonema, L: leafy plant, E: exospore, R: rhizoid, B: bud of leafy plant, FP: filamentous protonema.

not observed in this species. The initial cell of the leafy plant arises as a protuberance of a marginal cell of the thallose protonema. It has no connection with the rhizoidal area, as in *Sph. girgensohnii* and *Sph. squarrosus* (Noguchi³). The early stages of the development of the bud of leafy plant are similar to that of *Diphyscium fulvifolium*.

The initial cell divides repeatedly to form a cell mass, which differentiates later into a leafy plant (Fig. 27). After differentiation of the stem and leaves, new rhizoids begin to arise successively from the marginal cells of the thallose protonema. One or two leafy plants are born on a thallose protonema (Figs. 28, 31, and 32).

Summary

The protonema system of *Diphyscium fulvifolium* Mitt. consisted of both the filamentous and trumpet-shaped protonemata. The filamentous protonema developed poorly, and gave out only a few short branches and 1-3 clavate branches which later develop into trumpet-shaped protonemata. The terminal cell of the filamentous protonema was, though rarely, able to develop into trumpet-shaped protonema. In some cases, a trumpet-shaped protonema was successively produced on the preceding ones from an epidermal cell in the upper regions of the latter, forming a chain of two or three of such protonemata. The trumpet-shaped protonema is phototropic. The bud of leafy plants arose from certain basal cells or from certain epidermal cells of the upper part of the trumpet-shaped protonema. In the regeneration of this species the bud arises from the tip of clavate branches or from the filamentous protonema directly (Noguchi³), but this is not what we found in the present study on the sporelings.

The protonema system of *Sphagnum cuspidatum* Ehrh. consisted of both the filamentous and thallose protonemata. The filamentous protonema grown in the culture solution became long, while it attains a length of only 2 or 3 cells when cultured on agar or moistened porous plate or filter paper. In the porous plate culture the thallose protonema was ribbon-shaped, but sporelings cultured in nutrient solution, or on agar or wet filter paper produce a palmately lobed thallose protonema. The thallose protonema showed a phototropic character in both cases. *Sph. girgensohnii* was reported by Noguchi³ to form the second and the third, and even the fourth protonema. Such protonemata were not found in the present species. The rhizoids arose, from the endospore, the filamentous protonema, and marginal cells of the thallose protonema. One or two leafy plant were found on a thallose protonema.

The authors wish to express many thanks to Dr. A. Noguchi of Kumamoto University for his suggestions afforded for the present study. Thanks are also due to Miss Atsuko Yamasaki who helped the authors in various ways.

References

- 1) Campbell, D. H., Mosses and Ferns. New York (1918).
- 2) Müller, C., Musci in Engler and Prantl's Nat. Pflanzenfam. I. Aufl. Leipzig (1898).
- 3) Noguchi A., Journ. Hattori Bot. Lab. No. 19, 71 (1958).
- 4) Noguchi A., and Muraoka S., Kumamoto Journ. Sci., B. 2, 4: 118 (1959).

摘 要

西田雄行・斉藤真太郎： 蘚類胞子の発芽研究 II. イクビゴケおよびハリミズゴケ

イクビゴケの完成した原糸体は、糸状原糸体とろうと状原糸体から成り立つが、現在までの観察では第17図のごとく、それらの発生位置関係の異なる6つの型を見ることができた。このろうと状原糸体は向

日性を示しながら培養基から立ちあがった。多くの場合、その基部周縁部に数本の長い仮根を生じ、この付近の細胞から茎葉体の芽が生じたが、基部のみならずろうと状原糸体のろうと上縁部の表皮細胞からも生ずることがあった。しかし糸状原糸体の細胞や、こん棒状の枝の先端から直接に茎葉体の芽が生ずることはなかった。また第 14 図に見られるように、第 1, 第 2, 第 3 のろうと状原糸体が連続的に生じた場合もあった。このような例では第 1 の原糸体の上縁部の表皮細胞から茎葉体の芽が生じたが、第 2, 第 3 の原糸体からは培養期間中には芽を生じなかった。

次にハリミズゴケの原糸体は素焼板、寒天培養基、または汙紙上の培養では 2~3 個の細胞から成り立っていた。液状培養では非常に長い原糸体が生じる。この原糸体の頂端の細胞が大きくなり、かつ一定の分裂を行なって一層の細胞からなる葉状原糸体が生じる。この葉状原糸体は向日性が著しく、成長するにつれて、その先端部は培養基面から立ちあがった。液状培養、寒天培養、または汙紙上の培養のごとく湿気の多い場合には葉状原糸体は掌状になるが、素焼板上の培養ではリボン状になる。仮根は孢子、糸状原糸体および葉状原糸体の基部縁辺部のいずれからも生じるのは特徴的である。この培養では孢子をまいてから、約 2 カ月目に葉状原糸体の縁辺細胞に突起を生じ、この突起が細胞分裂を続けて茎葉体になる。1 個の葉状原糸体には 1~2 個の茎葉体が生育した。(島根大学教育学部附属中学校・島根大学文理学部生物学教室)

Effect of Indoleacetic Acid on Protein and Ribonucleic Acid Syntheses in Cultured Bean Germ-axes

by Mitsuo IZAWA*

Received July 29, 1960

Recently it has been reported that auxins can affect nucleic acid and protein metabolisms. Silberger and Skoog¹⁾ have found that added indoleacetic acid (IAA) stimulates the increase in ribonucleic acid (RNA) and desoxyribonucleic acid contents of cultured tobacco pith tissues. However, they have not touched on protein metabolism. On the other hand, Thimann and Loos²⁾ have observed that naphthaleneacetic acid stimulates protein production as well as water uptake in cultured tissues of potato tuber and of Jerusalem artichoke. In our laboratory, Oota and Osawa (unpublished data) have indicated that in cultured bean germ-axes added IAA (10 μ g./ml.) evokes net synthesis of RNA accompanying little increase of protein. Uemura (unpublished data) also observed that water uptake of the germ-axes was remarkably inhibited by the same concentration of IAA, and the IAA did not elevate RNA level but only prevented the decrease in the level. It is noted that these authors worked with the media in which the germ tissues could not elevate their protein levels.

In the present study the culture conditions which can give net synthesis of protein in germ-axes isolated from bean seeds was established, and the effect of IAA on the metabolism of protein and RNA were reinvestigated.

Materials and Methods

Germ-axes: Beans, *Vigna sesquipedalis*, stocked in a dark room for a year after harvest were used. The seeds were imbibed in 0.03% calcium hypochloride solution for 6 hours at 30°, and washed with sterilized water; germ-axes (exclusive of young leaves) were isolated with a sterilized razor blade in an aseptic room.

Tissue culture: Ten to 15 germ-axes in each 50 ml. Erlenmeyer flask containing 1 to 2 ml. sterilized culture medium (see the later description) were incubated at 30° in the dark for designated periods. The germ-axes were immersed partly in the medium. A series of preliminary experiments were carried out in search for culture conditions under which net synthesis of protein could be brought forth. White's medium containing 2% sucrose (referred to as *simple medium*)³⁾ was found to be efficacious for increasing length, fresh and dry weights of the germ-axes, and was unable to increase but rather decreased protein content (Fig. 1). Supplemented vitamin complex (B₁, B₂, B₆, C and nicotinamide; each 50 μ g./ml.), coconut milk** (3 to 10%)⁴⁾ or casein hydrolyzate (4 mg./ml.) to *simple medium* had no favorable effect. Only the decrease in protein level was arrested by the addition of coconut milk or casein hydrolyzate (Fig. 2). A little rise in protein level was caused by the addition of kinetin (10 μ g./ml.). A rise as high as 35% was induced by the addition of bean cotyledon hydrolyzate (see below) in 5 day period of culture (Fig. 2). Finally the following mixture (referred to as *control medium*) was attained which was able to

* Biological Institute, Faculty of Science, Nagoya University, Chikusa, Nagoya, Japan. 名古屋大学理学部生物学教室

** Coconuts were obtained from Hawaii by courtesy of Professor T. Mori.

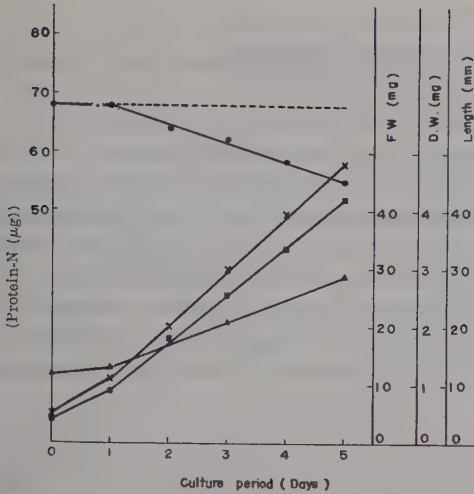


Fig. 1. Changes in fresh weight (F. W.), dry weight (D. W.), length and protein content of bean germ-axis cultured in *simple medium*. Values per axis plotted. —■—: F. W., —▲—: D. W., —×—: length, —●—: protein-N.

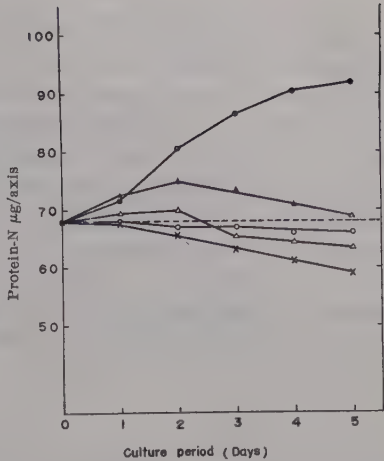


Fig. 2. Effect of various additions to *simple medium* on change in protein content of germ-axis. —×—: +vitamines, —Δ—: +coconut milk, —○—: +casein hydrolyzate, —▲—: +kinetin, —●—: +cotyledon hydrolyzate.

increase protein level by 50% in 5 days of culture: White's medium containing trace amounts of inorganic elements (Mn, Zn, B and I), sucrose 20 mg./ml., casein hydrolyzate 4 mg./ml., cotyledon hydrolyzate 10% and kinetin 10 µg./ml.; pH adjusted to 6.0 with 0.01N NaOH. *Control medium* supplemented further with auxin, IAA (1 µg./ml. of free acid; Merck), will be referred to as *IAA medium*.

Bean cotyledon hydrolyzate: Ten grams (fresh weight) of soaked bean cotyledons were homogenized with 50 ml. of 3% NaCl solution and centrifuged at 1,000×g (Kubota centrifuge, Model K-80) for 10 minutes. The supernatant was hydrolyzed with 6N HCl for 24 hours in a boiling water bath, neutralized with NaOH and made up to 100 ml. with water. One part of the hydrolyzate was added to 9 parts of the culture medium.

Subcellular fractionation: One hundred germ-axes were macerated with 30 ml. of 0.25 M sucrose and sea sand in a porcelain mortar. The brei was centrifuged at 1,000×g for 10 minutes to precipitate nuclear (*N*) fraction. The supernatant was centrifuged at 10,000×g (Servall centrifuge, Model SS-1) for 20 minutes and then at 105,000×g (Spinco ultracentrifuge, Model L, No. 40 rotor) for 90 minutes to obtain mitochondrial (*Mt*), microsomal (*Ms*) and supernatant (*Sp*) fractions. All procedures were performed at ca. 4°.

Length, fresh weight, dry weight, protein and RNA contents: Excepting the estimation of length and RNA content, the methods described previously were used⁵). Length of germ-axes was measured with an ordinary ruler. RNA was extracted by a combination of Schneider's and Ogur-Rosen's procedures⁶) and the absorbancy at 260 mµ was assayed with Beckman DU spectrophotometer. Nucleotide composition of RNA was estimated by Dowex 1 column chromatography⁷).

Results

Effect of IAA on daily changes in length, fresh weight, protein and ribonucleic acid contents of germ-axes.

In *control culture* (grown in *control medium*), the length, fresh weight and contents of protein and RNA of germ-axes change with culture period as shown in Fig. 3. The final length and fresh weight attained in 5 days were smaller by ca. 50% and greater by ca. 20% than those attained in the same culture period in *simple medium*, respectively (cf. Fig. 1 with Fig. 3), a remarkable increase in thickness of the tissues occurring in *control medium*. It was ascertained separately that these effects were due to the presence of kinetin in *control medium*, which probably promoted cell division⁸). Unexpectedly in *IAA culture* (grown in *IAA medium*) the fresh weight, length and protein content were little increased in the initial 2 days, and henceforth

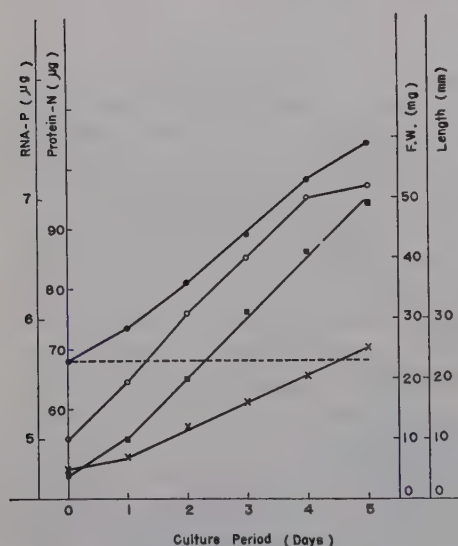


Fig. 3. Changes in F. W., length, RNA and protein contents of germ-axis cultured in *control medium*. —○—: RNA-P. See Fig. 1 for other symbols.

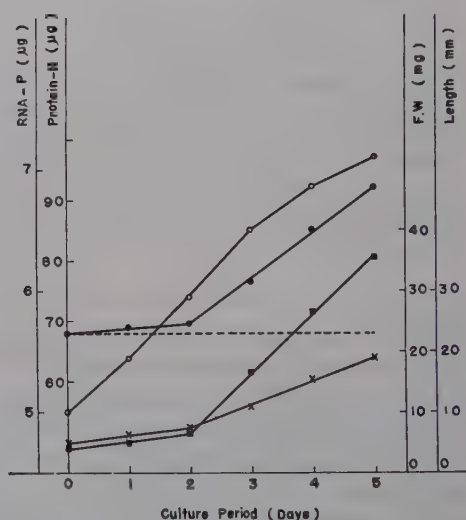


Fig. 4. Changes in F. W., length, RNA and protein contents of germ-axis cultured in *IAA medium*. See Figs. 1 and 3 for symbols.

they rose with normal velocity (cf. Fig. 3 with Fig. 4). Noticeable is that the RNA content alone increased normally irrespective of the presence of IAA added (Fig. 4). This initial inhibition of fresh weight increase or water uptake (cf. Fig. 1) in *IAA culture* might be attributed to the surplus IAA concentration in the tissues⁹). A good parallelism between water uptake and protein accumulation as shown in Fig. 4 suggests an intimate relation between these two processes.

Nucleotide composition of RNA and subcellular distribution of protein and RNA.

In connexion with this RNA synthesis not accompanying simultaneous accumulation of protein in *IAA culture*, it seemed possible that the accumulated RNA was such a kind as unavailable for protein synthesis. As indicated in Table 1, ribonucleic acid isolated from the whole tissues of *IAA culture* was not different in quality from RNA isolated from those of *control culture* as far as their nucleotide compositions were concerned. It was further suspected if, in *IAA culture* any dis-

Table 1. Nucleotide composition of RNA isolated from cultured bean germ-axes. Molar ratio (adenylic acid=10) shown.
C, cytidylic acid; U, uridylic acid; G, guanylic acid

culture condition	Culture period (days)	0			2			5		
		C	U	G	C	U	G	C	U	G
Control culture		9.8	10.9	13.7	9.6	10.8	13.7	9.5	11.0	13.8
IAA culture		—	—	—	9.9	10.9	13.8	9.8	10.8	13.9

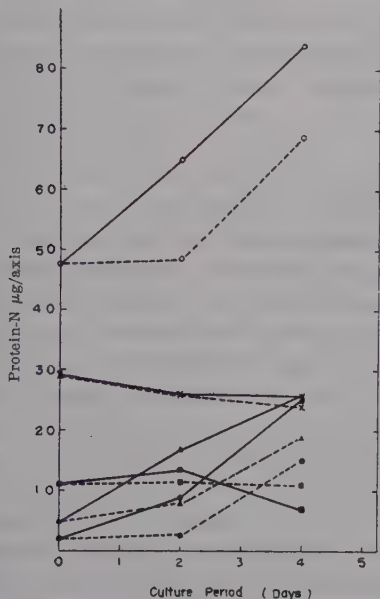


Fig. 5. Changes in subcellular distribution of protein with culture period. Solid and broken lines indicate *control* and *IAA cultures*, respectively. Values per axis plotted.
—○—: whole tissues, —●—: nuclear fraction, —▲—: mitochondrial fraction, —■—: microsomal fraction, —×—: supernatant fraction.

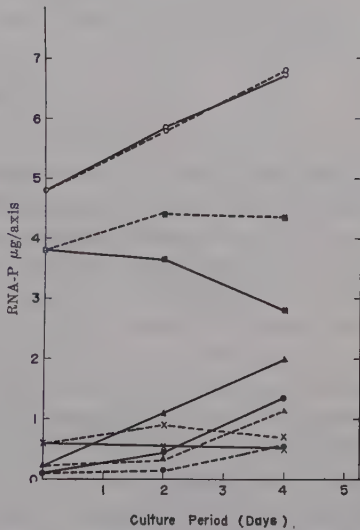


Fig. 6. Changes in subcellular distribution of RNA with culture period. See Fig. 5 for symbols and further explanations.

turbance in subcellular distribution of synthesized RNA might be responsible for the dissociation observed between the production of RNA and that of cytoplasmic protein. Daily changes in subcellular distribution of protein and RNA were examined for both *control* and *IAA cultures* (Figs. 5 and 6). In the initial 2 days period, the contents of protein* and RNA in *control culture* increased practically solely in larger granules, i.e., *N* and *Mt* fractions, but in *IAA culture* RNA alone, but little protein, were accumulated in smaller granules and soluble cytoplasm, i.e., *Ms* and *Sp* fractions. Later on, in *control culture* the above pattern of subcellular distribution of protein

* In the experiments of Figs. 3 and 4 the trichloroacetic acid (TCA) insoluble fraction, and in the experiments of Fig. 6 TCA, alcohol, alcohol-ether and hot perchloric acid insoluble fraction were taken as the protein fractions.

and RNA was not altered significantly, whereas in *IAA culture* protein as well as RNA increased not in *Ms* and *Sp* fractions but in *N* and *Mt* fractions. It must be mentioned that the rise in protein level of either *control* or *IAA culture*, if any, took place solely in fractions readily sedimentable (*N* and *Mt*) and that the rise always kept pace with the rise in RNA level of the same fractions. By the way, in 0 day-old germ-axes only 18% of protein and 8% of RNA were recovered in the *N+Mt* fractions, while in the 4 day-old *control culture* 60% of protein and 5% of RNA were in these fractions. In *IAA culture* the initial distribution of protein and RNA among subcellular components was retained unchanged for 2 days, followed by the same distribution pattern with that of aged *control culture*. According to Lund *et al.*¹⁰⁾, protein and RNA of mature cells of germinating corn roots are detected mostly in a fraction sedimentable at $5,000\times g$ for 15 minutes (heavy mitochondrial fraction). Preferential localization of protein and RNA in larger granules appears to be common in aged plant cells.

Discussion

No such stimulation of protein and RNA (net) syntheses could be induced in cultured bean germ-axes by exogenous IAA as was reported for isolated pith tissues by Silberberger and Skoog¹⁾ and for potato tubers by Thimann and Loos²⁾. Nor the stimulation of water uptake (assayed as the increase of fresh weight), as the most ordinary effect of exogenous auxins¹¹⁾, was given by added IAA in the present materials. The cause of the discrepancies between their results and the present ones is not yet clear. It should only be reminded here the remarks made by these American authors that the promotion of protein or nucleic acid production induced by exogenous auxins always keeps pace with stimulated water uptake.

Remarkable RNA accumulation without accompaniment of any rise in protein level in *Ms* and *Sp* fractions was demonstrated in the initial 2 days of *IAA culture*. It was found that RNA produced in this culture was not different in quality (nucleotide composition) from that produced in *control culture*. However, the nucleotide composition as assayed for RNA isolated from the whole tissues cannot inform us of exact nucleotide composition of individual subcellular component. Our recent analyses¹²⁾ have shown that nucleotide compositions of RNA's of *Ms* and *Sp* fractions isolated from germinating bean seedlings are significantly different from each other. It is desired to examine if any modification in RNA molecules in *Ms* and *Sp* fractions is caused by IAA added. At all events, it is remarkable that, in *Ms* and *Sp* fractions which are known as the central sites of cytoplasmic protein production¹³⁾, protein synthesis is inhibited by exogenous (perhaps surplus) IAA, RNA synthesis being little affected. RNA production independent of protein synthesis has also been reported to occur in the presence of chloramphenicol in bacteria¹⁴⁾. In this case RNA synthesized is known to be utilizable in protein synthesis after the reagent is removed from culture medium. Chloramphenicol seems to block the last step of protein synthesis, i.e., formation of peptide chain and/or polymerization of these peptide chains on microsomal particle¹⁵⁾.

It should also be noted that in the present culture conditions protein can accumulate only in the larger granules (*N* and *Mt* fractions) and the increase is always accompanied by RNA increase. It is difficult to say if the increase in protein levels of *N* and *Mt* fractions means the synthesis by these particles themselves, since Lund *et al.*¹⁰⁾ observed electron-microscopically that the heavy mitochondrial fraction prepared from mature corn root tissues was contaminated with many vesicles or

fragments of endoplasmic reticulum. On the other hand, we (Ichimura, Izawa and Oota, unpublished data) have noticed that in maceration process of the germinating bean cotyledons remarkable granulization of soluble cytoplasmic protein into dense particles that are co-precipitated with mitochondria occurs. This is likely due to acidification of the medium by the liberation of cell sap.

The author wishes to express his sincere gratitudes to Dr. Y. Oota for his guidance and advice.

Summary

1. Culture medium (referred to as *control medium*) in which the net syntheses of protein and RNA could occur was designed for isolated bean germ-axes.

2. The addition of IAA ($1 \mu\text{g./ml.}$) to *control medium* remarkably inhibited water uptake and protein synthesis for the initial 2 days, but gave no inhibitory influence on RNA synthesis. Nucleotide composition of RNA isolated from *IAA culture* (incubated in *control medium* plus IAA) agreed well with that of RNA isolated from *control culture* (incubated in *control medium*).

3. In *IAA culture* the increase of RNA in the initial 2 days occurred mainly in microsomal and supernatant fractions. But after this period, increase of both protein and RNA was restricted to nuclear and mitochondrial fractions. In *control culture* the rise in protein and RNA levels proceeded throughout the culture period only in nuclear and mitochondrial fractions.

References

- 1) Silberger, J., and Skoog, F., *Science* **118**: 443 (1953).
- 2) Thimann, K. V., and Loos, G. M., *Plant Physiol.* **32**: 274 (1957).
- 3) White, P. R., *A Hand Book of Plant Tissue Culture*, Lancaster (1943).
- 4) Steward, F. C., Bidwell, R. G. S., and Yemm, E. W., *Nature* **178**: 730 (1956).
- 5) Izawa, M., *Jap. Jour. Bot.* **16**: 135 (1958).
- 6) Oota, Y., and Osawa, S., *Experientia* **9**: 96 (1953).
- 7) Osawa, S., and Hotta, Y., *Protein, Nucleic Acid and Enzyme* (in Japanese), **3**, 40: 140 (1958).
- 8) Das, N. K., Patau, K., and Skoog, N., *Physiol. Plantarum* **9**: 640 (1956).
- 9) Izawa, M., *Bot. Mag. Tokyo* (in press).
- 10) Lund, H. A., Vatter, A. E., and Hanson, J. B., *Jour. Biophys. Biochem. Cyt.* **4**: 87 (1958).
- 11) Leopold, A. C., *Auxins and Plant Growth*, Berkeley (1955).
- 12) Ichimura, K., Izawa, M., and Oota, Y., *Plant and Cell Physiol.* **1**: 317 (1960).
- 13) Brachet, J., *Biochemical Cytology*, New York (1957).
- 14) Gale, F., *Advances in Prot. Chem.* **8**: 285 (1953).
- 15) Hopkins, J. W., *Proc. Nat. Acad. Sci.* **45**: 1461 (1959).

摘 要

井沢三生： ミトリササゲ培養胚におけるたん白質およびリボ核酸合成に及ぼす
インドール酢酸の影響

ミトリササゲ胚培養でたん白質やリボ核酸の増加する培養条件を見つけ、その条件下で吸水とたん白質およびリボ核酸の増加におよぼすインドール酢酸 ($1 \mu\text{g./ml.}$) 添加の影響をしらべた。インドール酢酸添加は培養の最初の2日間の吸水とたん白質の増加をほとんどとめるが、リボ核酸の増加には何ら影響を与えない。ただし、この最初の2日間、リボ核酸はマイクロソームおよび可溶性細胞質分画のみで増加する。そして全組織からとれたリボ核酸の塩基組成は対照のそれとちがいが無い。

対照培養では全期間を通じ、インドール酢酸添加培養では3日目以後、低速遠心 ($10,000 \times g$ まで) で沈でんされる部分で顕著なたん白質およびリボ核酸の増加が見られた。この現象は胚の吸水と関係があるように思われる。

An Ascospore Color Mutant of *Neurospora crassa**

by Kazuo NAKAMURA**

Received August 22, 1960

In *Neurospora crassa*, the present writer found one instance among stocks kept at Kyushu University, 4A, carrying a mutant gene affecting the ascospore color development. Crossing of the mutant with the wild-type of the opposite mating-type, a, produced perithecia which contained asci with two pairs of wild-type black and two pairs of tan-colored ascospores, thus making possible a direct analysis of the segregation of the spore color locus, symbolized as *tan-spore* or *ts*. Accordingly, this mutant may provide a good marker in analyzing the problems of the heterocaryon and of the genetic crossing-over. Similar segregations of ascospore color have been reported in homothallic^{1,2,3)} as well as in heterothallic fungi^{4,5)} other than *Neurospora*. In *Neurospora*, Stadler⁶⁾ reported a similar mutant, *asco*, with which he demonstrated the wide variation in frequencies of second-division segregation. The present paper deals with the linkage of *ts* to *inos* (37401) and with variation in recombination values due to strains mated to *tan-spore*.

Materials and Methods

Along with the mutant *ts*, the following five stock cultures of *Neurospora crassa* were employed: 37402a (*asco*), 8a (wild-type), P2a (wild-type), B135a (*osmotic*), and a linkage tester LT2a. The present B135a is an isolate from successive back-crosses of the original mutant to St. Lawrence wild-type (Dr. Perkins' personal communication). For convenience this symbol B135a will be used in this paper.

Westergaard and Mitchell's⁷⁾ minimal agar medium in Petri-dishes was used for crossings, and was supplemented appropriately when nutritional mutants were involved. The initial pH of the medium was adjusted to about 6.2. Crosses were made by applying conidial suspension in sterile distilled water to the protoperithecia developed by *ts*, excepting a cross involving LT2a which was made possible by simultaneous inoculation with *ts* because of the retarded development of the protoperithecia. All cultures were incubated at 25° in darkness. Seven to eight days after the application of conidial suspension some of the asci in the developed perithecia began to segregate in color. Thereafter, difference in color between the two kinds of spores became increasingly distinct day by day in most of the asci. These two kinds of spores were readily distinguishable till the spores were expelled, though tan spores became somewhat darker with age. Usually 11 days after the fertilization, perithecia were dissected for microscopical examinations. In the cross involving LT2a the dissection was carried out two weeks after the inoculation. Ascus clusters were mounted in glycerine-water mixture, this mounting medium having been proved to be most satisfactory to distinguish the spore color and to preserve the preparations for a long period.

Results

1. *The gene ts*. After receiving a stock labelled 'wild-type 4A' through Dr. Y. Kobayashi, National Science Museum, Tokyo, in 1952, the stock has been maintained

* Contribution from the Department of Biology, Faculty of Science, Kyushu University, No. 106. Aided in part by a Grant-in-Aid for Fundamental Scientific Research from the Ministry of Education, Japan, No. 407112.

** Cytogenetics Laboratory, Department of Biology, Faculty of Science, Kyushu University, Fukuoka, Japan.

by mass transfers by Dr. H. Ono, General Education Department of Kyushu University. When this 4A was first crossed to 8a in our laboratory in 1953, the cross revealed clear segregation into four wild to four tan spores. The asci were classifiable into the well known six spore patterns of a monogenic segregation: two patterns of

Table 1. Frequency of spore patterns resulted from the cross *ts* × 8a.

Spore pairs from the top of ascus	Spore patterns						Total asci
	I	II	III	IV	V	VI	
1, 2	+	<i>ts</i>	+	<i>ts</i>	+	<i>ts</i>	
3, 4	+	<i>ts</i>	<i>ts</i>	+	<i>ts</i>	+	
5, 6	<i>ts</i>	+	+	<i>ts</i>	<i>ts</i>	+	
7, 8	<i>ts</i>	+	<i>ts</i>	+	+	<i>ts</i>	
a)*	193	188	91	80	79	99	730
	(52.2%)		(47.8%)				(100%)
b)*	16	18	9	7	6	3	59
	(57.6%)		(42.4%)				(100%)
Total	209	206	100	87	85	102	789
	(52.6%)		(47.4%)				(100%)

* a) stands for regular asci with four dark and four tan spores and b) for irregular asci with one extra-pale spore.

first-division segregation and four of second-division segregation (Table 1; Figs. 1, 2). This cross gave 47.4% second-division segregation, indicating that the gene *ts* is located at a distance of 23.7 units from the centromere (Table 3). No evidence for biased segregations was obtained in this cross (for patterns I and II, $\chi^2=0.022$, D.f.=1, $P>0.80$; and for patterns III-VI, $\chi^2=2.449$, D.f.=3, $P>0.30$). The gene *ts* exerts no effect upon the size and shape of the spores, but it proved to be lethal at a certain stage in the spore maturation. So, attempts to germinate any of the tan spores, either those dissected from the asci or those which had been naturally expelled from the asci onto the agar medium, have failed to date, thus making it impossible to isolate a further new *ts* stock.

It is well known in *Neurospora* that the sporadic inclusion of immature or aborted spores in the ascus results in aberrant spore patterns with regard to spore pigmentation. Such aberrant spores could be attributed to chromosomal translocations⁸), to gene mutations⁹), or to a kind of gene competition in the course of spore maturation¹⁰). Further, such aberrant spores occurred under certain cultural conditions¹¹). In the present cross *ts* × 8a, as well as in the other crosses involving *ts*, a few asci were found to show such aberrant patterns as three black to five pale-colored spores, two black to six pale spores, and so forth. This is apparently due to the occasional failure of the normal pigmentation in the spores expected otherwise to develop into the black wild-type spores. This failure in pigmentation was noted in approximately 4% of the non-*ts* spores, occurring at random in position. For example, in a cross *ts* × 8a frequency of the second-division segregation was 47.8% in asci showing regular 4:4 segregation, and was 42.4% in asci with one extra-pale spore (Table 1). Discrepancy between these two frequencies was not significant ($\chi^2=2.331$, D.f.=1, $P>0.10$). Asci which contain one pair or more of extra-pales are not identifiable as to the segrega-



Figs. 1-3. Segregation of spore color loci in *Neurospora crassa*. 1. Cluster of asci from the cross $ts \times +$, $\times 100$. 2. Asci from the cross $ts \times +$, two on the left showing first-division segregation and two on the right showing second-division segregation, $\times 600$. 3. Asci from the dihybrid cross $ts \times usco$, from the left, a tetra-type, two parental ditypes, and a non-parental ditype ascus, $\times 600$.

tion pattern, and they are excluded from statistical records in the present article.

2. *Independence of *ts* from *asco**. A lysine-requiring mutant of *Neurospora*, 37402, was previously reported to carry a gene *asco*, which produces colorless spores and is located on linkage group 6⁸). To test the allelism and linkage relationship of *ts* to *asco*, *ts* was crossed with *asco* (37402a). If these two genes are not allelic, three types of asci, parental ditype (PD), non-parental ditype (NPD), and tetratype (T), should be produced, viz., 4(+ *ts*): 4(*asco* +), 4(+ +): 4(*asco ts*), and 2(+ +): 2(+ *ts*): 2(*asco* +): 2(*asco ts*). In reality this was the case. However, it was impossible to distinguish all the four genotypic spore types, because spores carrying *asco* yield the same colorless spores irrespective of the presence or absence of the gene *ts*, that is, this is a case of hypostasis of *ts* to *asco*. Thus there were distinguished phenotypically three kinds of asci: ascus with two pairs of tan spores and two pairs of colorless ones (PD), ascus with two pairs of black wild type spores and two pairs of colorless ones (NPD), and ascus with one pair of wild-type, one pair of tan, and two pairs of colorless spores (T) (Fig. 3). Observed numbers of these three types of asci were as follows:

PD	NPD	T	Total
133	98	173	404.

If the two loci segregate independently from one another, two kinds of ditype tetrads should be equally frequent¹²). But, the difference between the number of PD and that of NPD asci was significant ($\chi^2=5.303$, D.f.=1, $0.02 < P < 0.05$). There was, however, no way of distinguishing a tan spore from a young wild-type spore in a few asci which were in maturation process. Thus an ascus with four tan and four colorless spores could be either a NPD or a T as well as a PD ascus at its maturity, which may result in an excess of PD, no definite evidence for linkage between *asco* and *ts* being manifested.

3. *Locating *ts* on linkage group 5*. To identify linkage group on which *ts* is located, this mutant *ts* was crossed with the linkage tester stock LT2a in which all the seven linkage groups were marked with one mutant locus each (cf. Table 2). Random spores from this cross were germinated and 100 spores were isolated. All the germinating spores from such a cross should carry the wild-type allele of the *ts*. If any mutant locus is independent of *ts*, half the germinating spores will be mutant. And, if a certain mutant locus links with *ts*, more than 50% of the germinating spores will exhibit the mutant character. Of 100 isolates, 96 revealed inositol-requirement, thus distance between *ts* and *inos* being estimated as 4.0 units. Whereas, other genes exhibited the mutant character in about 50% of the isolates (Table 2). This indicates that *ts* is located on linkage group 5. Centromere distance of *inos* is 26.2 units¹³); *ts* is probably located on the right arm together with *inos*.

4. *Variation in genetic distance of *ts* from centromere*. As generally accepted, the genetic distance of a locus from the centromere is estimated by halving the frequency of second-division segregation. And this procedure has been taken for granted to give a genetic distance particular to a given locus. Recently, however, Stadler⁶) has called the writer's attention to his finding that the value estimated by this procedure for *asco* varies widely according to the stocks combined in crossing—5 to 29 units.

In the present case of *ts*, the value 23.7 was obtained for the cross *ts*×8a. Further crosses of *ts*×P2a, *ts*×B135a, and *ts*×LT2a gave the values 13.1 (13.0 and 13.1), 14.1 (13.3 and 14.9), and 30.4 (30.0 and 31.1), respectively, two figures in parentheses indicating actual values recorded from two Petri-dishes (Table 3). Although the value varied within such a wide range, according to the stocks employed, the

Table 2. Analysis of the cross of *ts* × tester stock LT2a.*

Marker gene	Linkage group	Expectation on no linkage with <i>ts</i>	Recombinant spores obs.	% recomb.	χ^2 test P
<i>al-2</i> (15300)	1	50	51	51	>0.05
<i>fl</i> (fluffy)	2	24**	19	39.6	>0.05
<i>sc</i> (5801)	3	50	48	48	>0.05
<i>pan</i> (5531)	4	50	64	64	0.005-0.11#
<i>inos</i> (37401)	5	50	4	4	<0.001
<i>ylo</i> (Y30539y)	6	25.5**	27	52.9	>0.05
<i>nt</i> (C86)	7	50	59	59	>0.05

* Details of the seven marker genes are described in Barratt *et al.*¹³⁾.

** Since *fl* is hypostatic to *sc*, and *ylo* to *al-2*, *fl* and *ylo* characters were determinable in *sc*⁺ and *al-2*⁺ isolates, respectively.

An excess of recombinant may be attributed to low viability of the mutant.

Table 3. Frequencies of second-division segregation of *ts*, analyzed in asci from crosses between *ts* and various stocks.

Stocks crossed	Total asci	Second-division segregation		Centromere- <i>ts</i> distance	
		asci	(%)	value	range*
LT2a	1082	658	60.8	30.4	28.4-32.3
8a	789	374	47.4	23.7	21.6-25.8
B135a	1718	486	28.3	14.1	12.4-16.0
P2a	1233	322	26.1	13.1	11.4-15.0

* Ranges were obtained from confidence limits curves (cf. Barratt *et al.*¹³⁾).

values estimated in the replicates with a given stock revealed only a restricted variation.

Discussion

i) *Origin of the tan-spored mutant.* Strain 4A was introduced to Japan by Prof. D. Moriwaki of Tokyo Metropolitan University from the California Institute of Technology in 1950. The writer's stock *tan-spore* originated from subcultures of that strain obtained through the courtesy of the Nagao Institute, Tokyo. The original wild-type 4A has been maintained in Japan in substock cultures kept at Osaka University, the National Institute of Genetics, and the Nagao Institute. Hence, it is highly probable that spontaneous mutation from wild to *ts* might have occurred in a wild-type mycelium, forming a heterocaryon¹⁴⁾. Then, the heterocaryon containing *ts* and wild-type nuclei might have become a homocaryon in respect to *ts* by some selective advantage of *ts* nuclei over wild-type nuclei or by chance selection during serial transfers. In support of this inference, Wilson³⁾ reported a case of heterocaryosis for an ascospore character due to a mutation in a single ascospore isolate of *Gelasinospora calospora*; Ryan and Lederberg¹⁵⁾ presented evidence for homocaryonization in an artificial heterocaryon of a *leucineless* mutant of *N. crassa*, and Olive¹⁾ reported a similar case in a grey-spored mutant of *Sordaria fimicola*.

ii) *Variation in cross-over frequency.* Cross-over frequency in the region between *ts* and the centromere varied widely according to the stocks combined in crossing (Table 3). Similar results have been reported in linkage group 6 by Stadler⁶⁾ and in

linkage group 1 by de Serres (cf. Giles *et al.*¹⁶). According to these studies it may be taken for granted that variation in cross-over frequency in a given region may happen universally in this fungus. Stadler⁶) suggested the presence of a large number of heritable factors influencing cross-over frequency in *Neurospora* stocks. In this connection, it may be of interest that the cross-overs increase with serial successive back crosses (Nakamura, unpublished). According to Giles *et al.*¹⁶) the differential survival of segregants also affects the cross-over frequency. Doermann¹⁷) reported a translocation in linkage group 1 which reduced cross-over frequency. Thus, the variation in cross-over frequency in a given region can be interpreted as being due to the heterogeneity in genic and/or chromosomal back-ground of the stocks.

Concluding this paper, it must be emphasized that the cross-over data should be always carefully considered in connection with the strains employed in crossing.

Summary

- 1) In *Neurospora crassa*, an ascospore color mutant *ts*, which produces tan-colored spores was found to occur spontaneously.
- 2) Linkage test crosses revealed that *ts* is located on the right arm of linkage group 5.
- 3) Genetic distance from centromere to *ts* was estimated in four different crosses, to have the values 13.1, 14.1, 23.7, and 30.4, respectively.

The writer wishes to express his appreciation to Prof. T. Haga under whose guidance this research has been carried out. The writer further wishes to acknowledge his indebtedness to Dr. D. D. Perkins, Stanford University, for stock and advice and to Dr. D. R. Stadler, University of Washington, for mutant *asco*.

References

- 1) Olive, L. S., Amer. Jour. Bot. **43**: 97 (1956). 2) Heslot, H., Paper presented at 9th Intern. Congr. Genetics, Bellagio, Italy (cited after Olive 1956). 3) Wilson, G. B., and Alexopoulos, C. J., Mycologia **48**: 685 (1956). 4) Zickler, H., Planta **22**: 573 (1934). 5) Bistis, G., Bull. Torrey Bot. Club **83**: 35 (1956). 6) Stadler, D. R., Genetics **41**: 528 (1956). 7) Westergaard, M., and Mitchell, H. K., Amer. Jour. Bot. **34**: 573 (1947). 8) McClintock, B., *ibid.* **32**: 671 (1945). 9) Garnjobst, L., and Tatum, E. L., *ibid.* **43**: 149 (1956). 10) Lindegren, C. C., Zts. indk. Abst. Vererbungsl. **68**: 331 (1935). 11) Weijer, J., Genetica **27**: 173 (1954). 12) Perkins, D. D., Genetics **38**: 187 (1953). 13) Barratt, R. W., Newmeyer, D., Perkins, D. D., and Garnjobst, L., Adv. in Genetics **6**: 1 (1954). 14) Beadle, G. W., and Coonradt, V. L., Genetics **29**: 291 (1944). 15) Ryan, F. J., and Lederberg, J., Proc. Nat. Acad. Sci. **32**: 163 (1946). 16) Giles, N., de Serres, F., and Barbour, E., Genetics **42**: 608 (1957). 17) Doermann, A. H., Arch. Bioch. **5**: 373 (1944).

摘 要

中村和郎：アカパンカビの胞子の色の突然変異株

- 1) アカパンカビの子嚢胞子色に影響をおよぼす突然変異遺伝子が見いだされ、*ts* と名づけられた。
- 2) 連鎖群検定交配の結果、*ts* は第5連鎖群の右腕にあることがわかった。
- 3) 動原体—*ts* 間の遺伝学的距離は4種類の保存株との交配で変動を示し、それぞれ13.1, 14.1, 23.7 および 30.4 であった。(九州大学理学部生物学教室)

Synergistic Effect of Indoleacetic Acid and Kinetin on the Primary Thickening of Pea Stem Segments

by Tohru HASHIMOTO*

Received August 29, 1960

Since the discovery of auxins as growth regulators of plant stems, many studies have been accumulated on the growth of stem in connection with auxin action. However, attention of investigators has generally been directed almost to extension growth, i.e. increase in length of the stem, but not to thickening growth or increase in thickness. Although, the thickening of stems has been dealt with by some investigators including Snow, Söding, and Avery *et al.* (refer to Söding¹), and Meyer and Anderson²), their studies have been restricted to the secondary thickening growth in connection with the cambium activity of stems, and no information is available on the control of a primary thickening in stems by a growth substance or substances.

In the shoot of plants the apical meristem produces young cells. The cells, being at first very small, show an increase in size. If the increase takes place in the longitudinal direction, this process results in extension growth of the stem, and if in the lateral direction, an increase in thickness of the stem appears. It has been established that the former process is regulated by auxins, whereas no substance is thus far known that controls the latter, although it is probable that a substance or substances control the increase in cell size in the lateral direction of the stem. The present paper describes that kinetin exerts in the pea stem such an action as the presumable substance in the presence of indoleacetic acid (IAA).

Experimental

As the material stem and petiole segments of *Pisum sativum* L. var. Alaska were used. The seedlings were cultivated in pots containing field soil in a greenhouse at 15-25°. The seedlings were harvested when their petioles attached to the sixth nodes were upright, the leaflets of the sixth leaf were not completely unfolded, and the sixth internode did not appear yet. A single segment of 5.2 mm. in length was cut from the fifth internode ca. 5 mm. below the apical node and from the middle portion of the uppermost petiole. Two leaf discs, 5.0 mm. in diameter and not including the midrib, were punched out with a cork borer from one leaflet. The stem segment from the seventh internode was likewise prepared when the internode was at the same growth stage as that of the above-mentioned fifth internode. All these materials were at a stage of vigorous growth. More detailed description on the materials was presented in the previous paper³).

The three sorts of materials thus prepared were floated on 10 ml. of culture medium for incubation. In the case of leaf discs care was taken to place the discs upper surface up. As the medium, was used Boysen-Jensen's culture solution containing 2 per cent sucrose with or without the addition of IAA, kinetin or the combination of the two. The incubation was performed for 18 hours under about 1,000 lux at 25°. The illumination was supplied by white fluorescent tubes (Toshiba Co.).

After the incubation, measurements were made of the length, diameter and fresh

* Biological Laboratory, Musashi University, Nerima-ku, Tokyo, Japan.

weight of the segments, and of the area and fresh weight of the leaf discs. The length and diameter of the segments were determined with a low power binocular microscope, and the fresh weight, with a micro-torsion balance. The area of leaf discs was estimated from weight of cut shadow graphs on uniform printing paper.

For microscopic observation, 6 stem segments of average size were selected from each of the treated and control lots as well as from the materials before incubation and were fixed in FAA, dehydrated in tertiary butyl alcohol series and embedded in paraffin according to Sass's text-book⁴). Serial cross sections, 15 μ in thickness, were prepared and were stained with Heidenhain's iron haematoxylin. This dehydration method was recommended as an ideal one causing only a very small shrinking of samples.

Results

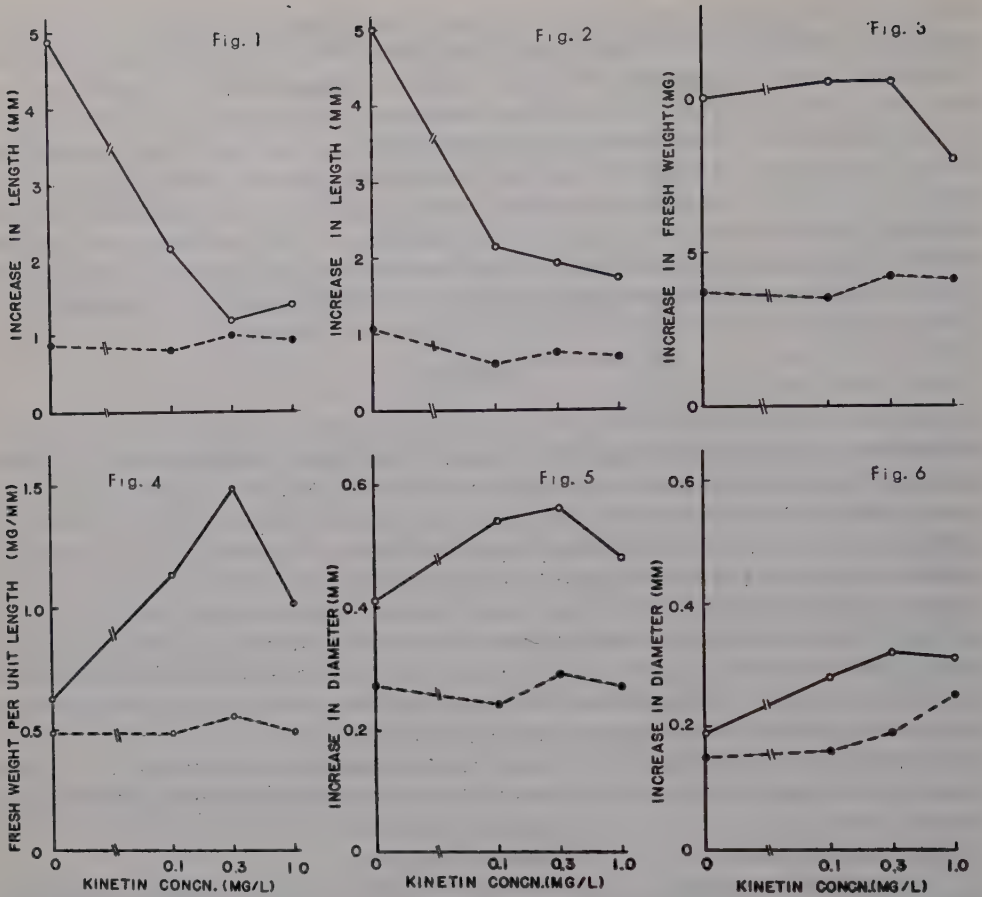
IAA-induced increase in length of the stem and petiole segments is inhibited by the addition of kinetin. As seen from Fig. 1 and Fig. 2, this inhibition by kinetin increases with the increasing concentration of kinetin and, in the stem, almost completely nullifies the action of 10 ppm IAA (the optimum concentration for the extension growth) at concentrations above 0.3 ppm of kinetin. Although in the petiole the maximum inhibition is smaller than in the stem, a similar trend is observed in the curves. This antagonism of kinetin and IAA in the extension growth is quite in agreement with de Ropp's result⁵) obtained with the fragments of *Helianthus* hypocotyl. The increase in fresh weight which occurs as a result of the IAA application, on the other hand, is not inhibited by kinetin even at 0.3 ppm, a concentration high enough to abolish the IAA-induced increase in length.

When a fresh weight per unit length of stem segments is computed, it is easily found to be much larger in IAA-kinetin-treated segments than in IAA-treated or in kinetin-treated ones (Fig. 4). The increase in fresh weight per unit length probably means that the thickening of the segments took place.

The direct determination of the diameter of the stem manifests, as shown in Fig. 5, that an increase in thickness is really caused. The optimum concentration of kinetin is 0.3 ppm at the dose of 10 ppm IAA, and at concentrations above 0.3 ppm the effect of kinetin rather decreases. To be noted especially is that in the absence of IAA this effect of kinetin is never observed and kinetin requires IAA to induce the thickening growth of the stem. Fig. 6 demonstrates that this effect of kinetin and IAA is reproduced in the petiole segments as well. In Fig. 7 the photographs of the kinetin-IAA-treated and untreated stem segments are shown.

The growth in thickness of the stem and petiole segments is slightly brought about by IAA alone, but when kinetin which is quite ineffective in itself is added together with IAA, a remarkable increase in the thickness is obtained. IAA and kinetin act antagonistically on the elongation, but synergistically on the thickening of stem and petiole. This fact suggests that the stem and petiole have different properties for growth between the longitudinal and transverse directions.

The expansion of leaves is stimulated by either kinetin^{6,7,8}) or IAA³). A synergism between these substances has been also reported in the expansion of radish leaf discs⁹). As shown in Table 1, the corresponding result was obtained in the pea leaf discs, too. In this experiment IAA was added at 10 ppm, the optimum concentration for the expansion of the leaf discs³). Even when solely applied, kinetin is effective. This can be considered, judging from the result obtained with the thickening of the stem segments, that kinetin exerts the action in combination with endo-



Figs. 1, 2. Effect of kinetin on the IAA-induced increase in length of the stem and petiole segments.

Fig. 3. Effect of kinetin on the increase in fresh weight of the stem segments in the presence and absence of IAA. The initial fresh weight of a stem segment is 6.41 mg.

Fig. 4. Effect of kinetin on the increase in fresh weight per unit length of the stem segments in the presence and absence of IAA. The initial fresh weight per unit length of a stem segment is 1.23 mg.

Figs. 5, 6. Effect of kinetin in the presence and absence of IAA on the increase in diameter of the stem and petiole segments. Their initial diameters are 1.28 and 1.12 mm., respectively. Figs. 1-6. The solid and broken lines indicate the data in the presence and absence of IAA, respectively. IAA concentration administered was always 10 ppm. The incubation was made for 18 hours under ca. 1,000 lux white fluorescent light at 25°.

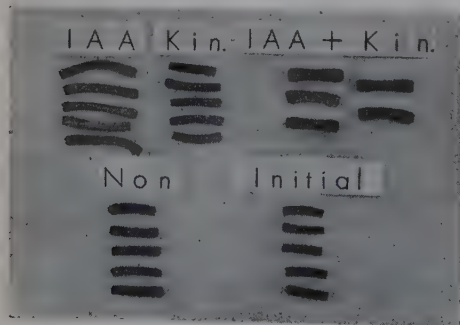


Fig. 7. The photographs of IAA-treated, kinetin-treated and IAA-kinetin-treated stem segments. The photographs of untreated stem segments and the segments before incubation are shown for comparison. A distinguished thickening is seen in the IAA-kinetin-treated segments. The concentrations of IAA and kinetin administered were 10 and 0.3 ppm, respectively.

Table 1. The Synergistic effect of kinetin and IAA on the expansion of pea leaf discs. The concentrations of kinetin and IAA are 1 and 10 ppm, respectively. Each figure indicates the increase as per cent of that of control during 20 hour culture.

Treatment	Fresh weight	Area
Control	100	100
Kinetin	181	121
IAA	172	129
Kinetin+IAA	208	138

genous auxin, at least, indoleacetic acid therein.

For the purpose of obtaining more detailed information about the thickening of the stem induced by simultaneous administration of kinetin and IAA from the histological viewpoint, a microscopical observation was made on the serial cross sections of the stem segments. Using the microscope equipped with a micrometer the diameter and the number of cell layers along the diameter of the treated segments were determined in comparison with those of control and those before incubation. In the case of the segments from the seventh internodes the distance from the epidermis to the innermost layer of the pith and number of layers of cells therein were adopted for measurement, since the segments have the pith-cavity in the centre. As seen in Fig. 8, the cross section of the stem is a square with large vascular bundles in the corners. The measurement was carried out through the interfascicular region as indicated by the line in the figure.

To the microscopic observation six stem segments were submitted from each lot, and of the serial sections of each segment were examined the following ten sections: 3 sections from the part near one end of the segment, 3 sections from the part near

Table 2. The results of the microscopic examination on the cross sections of IAA-kinetin-treated and untreated stem segments, and of those before incubation. The culture of the segments was made for 18 hours under ca. 1,000 lux white fluorescent light at 25°. The concentrations of IAA and kinetin administered were 10 and 0.3 ppm, respectively.

		IAA+kinetin	Untreated	Before incubation
5th internode	Diameter (mm.)	1.12 \pm 0.03*	0.87 \pm 0.02	0.79 \pm 0.01
	No. of layers	25.5 \pm 0.7	25.3 \pm 0.5	25.3 \pm 0.7
7th internode	Diameter (mm.)	1.28 \pm 0.03	1.10 \pm 0.03	
	Distance from epidermis to innermost layer of pith (mm.)	0.496 \pm 0.017	0.378 \pm 0.013	
	Diameter of pith-cavity (mm.)	0.316 \pm 0.039	0.296 \pm 0.033	
	No. of layers from epidermis to innermost layer of pith	12.2 \pm 0.36	12.3 \pm 0.34	

* The figures connected by \pm symbol, which were calculated from the standard error of each mean value, indicate 0.95 confidence intervals.

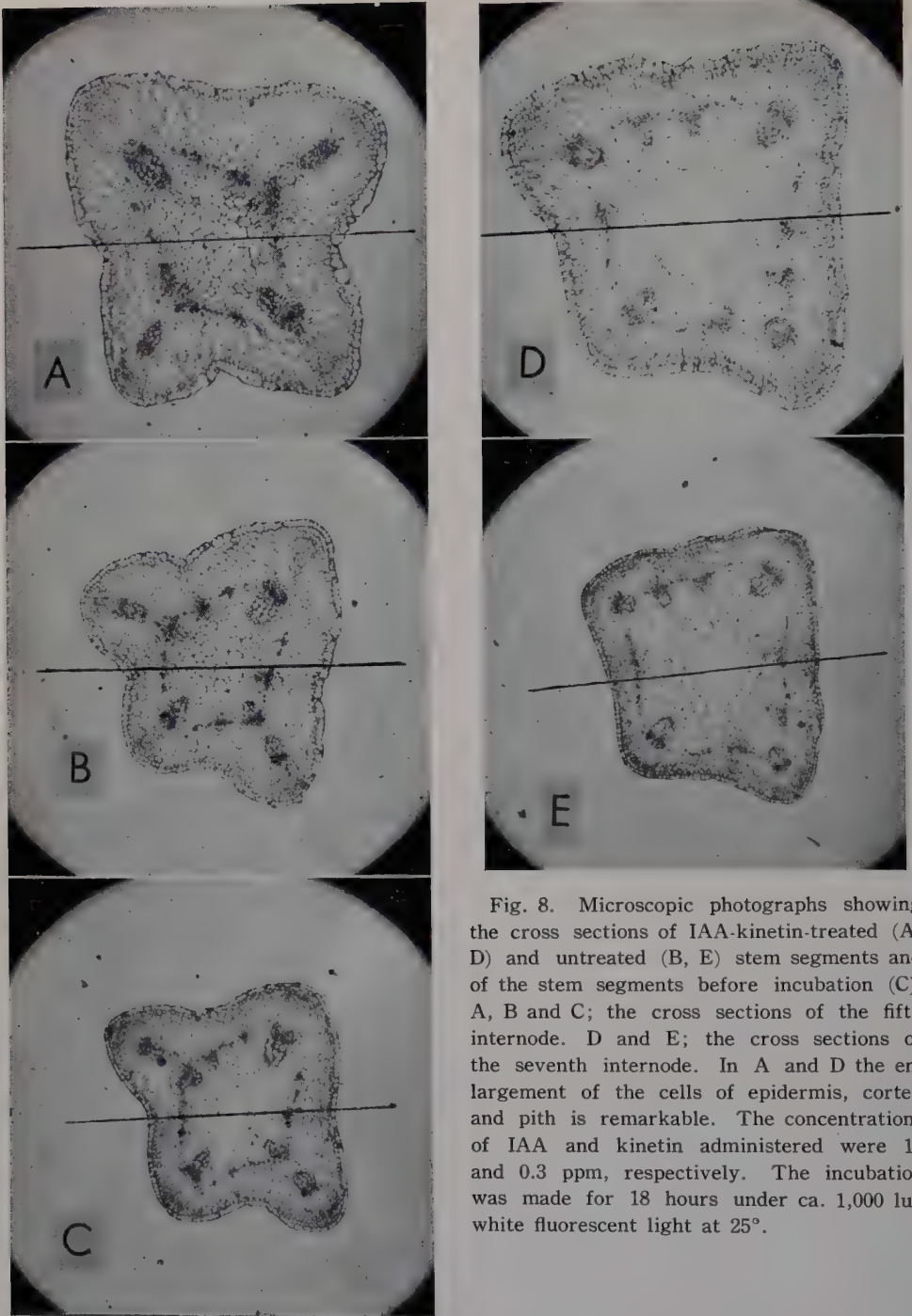


Fig. 8. Microscopic photographs showing the cross sections of IAA-kinetin-treated (A, D) and untreated (B, E) stem segments and of the stem segments before incubation (C). A, B and C; the cross sections of the fifth internode. D and E; the cross sections of the seventh internode. In A and D the enlargement of the cells of epidermis, cortex and pith is remarkable. The concentrations of IAA and kinetin administered were 10 and 0.3 ppm, respectively. The incubation was made for 18 hours under ca. 1,000 lux white fluorescent light at 25°.

the other end, and 4 sections from the middle part, each section being selected every 17 to 20 sections. Thus an unbiased selection of examined sections was secured.

The data of the microscopic examination are shown in Table 2. As seen from the table, the thickness of the stem, expressed in terms of the diameter, increases during the 18 hour incubation period even in the control segments, but in the kinetin-IAA-treated segments the increase in thickness is much more remarkable. As is observed in Fig. 8, the epidermis, cortex and pith take part in the thickening.

The number of the cell layers, on the other hand, changes during the experimental period neither in the treated nor in the control segments as compared with that of the segments before incubation. Furthermore, no microscopical figure of cell division was seen throughout the section. The above observations indicate that no appreciable number of cell division took place in the incubation period of 18 hours. These findings are the case in the segments not only from the fifth internode but also from the seventh one. Accordingly it is concluded that the increase in thickness of the kinetin-IAA-treated stem segment is, under these experimental conditions, due to an increase in cell size but not in cell number.

Discussion and Conclusion

It was found that pea stem and petiole segments responded to a simultaneous treatment of kinetin and IAA, though not to either of them alone, by an increase in thickness, without any detectable amount of cell division. This is the first* report that the primary thickening of the stem is induced by growth substances.

Jablonski and Skoog¹¹⁾ and Naylor *et al.*¹²⁾ have reported that in tobacco pith culture IAA brings about a cell enlargement entirely unaccompanied by cell division. Thereafter, the Wisconsin group including Skoog and Miller has found¹³⁻¹⁶⁾ that the addition of kinetin together with IAA to the culture induces cell division. These reports seem to be in discrepancy with the present result. However, if it is considered that they used isolated pith tissues and moreover cultured them as long a period as 3 to 24 days, the above seeming discrepancy in results may be readily explained. In addition Kuraishi and Okumura have reported⁷⁾ that the promotion of leaf growth by kinetin is ascribed to cell enlargement. It can be assumed that kinetin has two aspects of action such as cell enlargement and cell division.

To be considered here is the question whether the aspect of the thickening mentioned above really exists in normal plants, under the natural conditions. Esau states¹⁷⁾, referring to the work of Troll and Rauh with herbaceous dicotyledons, that the primary thickening of the axis occurs through cell division and cell enlargement. In dicotyledons and gymnosperms this growth may be rather diffuse, or more or less restricted to the pith or cortex. Ball has found¹⁸⁾ with the stem of *Lupinus albus* L. that an increase in diameter of the pith which is associated with a thickening of the subapical region of the stem is brought about mainly by an increase in cell size, and an increase in cell number is somewhat less important as a cause of the thickening. These findings evidence that such an aspect of the thickening of stems as due to the increase in cell size really forms an integral part of the process of stem growth.

In fact the region of the stem from which the segment has been excised in the present experiment is *in situ* in the state of thickening growth as well as of extension growth. When it is excised and cultured on the medium containing sucrose and some minerals essential for plant life, and even when IAA is added, the segment does not show thickening, unless kinetin is added to the culture medium. This fact suggests that this region of the stem makes the growth in thickness by the supply

* Recently Katsumi reported¹⁰⁾ a quite similar result with etiolated pea epicotyl segments.

of kinetin or kinetin-like substance(s) *in situ*.

Popp, long ago, reported¹⁹⁾ that a plant receiving higher intensity light produced a thicker stem than that receiving lower intensity light. We also have observed²⁰⁾ in the dark-grown seedling of broad bean, that brief irradiation by low intensity of incandescent light causes a thickening of the stem of the seedlings as well as an expansion of the leaf. These phenomena are well-known as a feature of prevention of etiolation by light.

Kinetin has such biological actions quite similar to light as stimulation of leaf expansion⁸⁻⁹⁾, unbending of the apical hook of dark-grown seedlings⁶⁾, inhibition of IAA-induced elongation of the stem⁵⁾, promotion of IAA-induced elongation in *Avena* coleoptiles^{21, 22)}, fulfilment, although partial, of nonphotosynthetic light requirement for multiplication of *Lemna minor*²³⁾, and breaking of seed dormancy⁶⁾. In the present study a new action of stimulating the thickening of the stem was added to kinetin as its effects similar to light actions. Although a general occurrence of kinetin in higher plant tissues is thus far not evidenced, it is probable that kinetin or kinetin-like substance(s) plays an important rôle in the thickening of stems.

I wish to express my thanks to Associate Prof. T. Yamaki for his guidance and to Dr. N. Hara for his generous help in making the microscopic preparation. This experiment was done at the Biological Institute, College of General Education, University of Tokyo.

Summary

The primary thickening of the stem segment of pea (*Pisum sativum* L. var. Alaska) has been studied from the viewpoint of growth substance and histology. It has been found that the thickening is induced by the simultaneous administration of IAA and kinetin, but not by either of them given separately, and that the thickening is due to an increase in cell size of the epidermis, cortex and pith, unaccompanied by an increase in cell number. It is discussed that the thickening of the intact stem is regulated by kinetin or a kinetin-like substance(s) in the plant in co-operation with endogenous auxin.

References

- 1) Söding, H., Die Wuchsstofflehre, Georg Thieme Verlag, Stuttgart (1952).
- 2) Meyer, B. S., and Anderson, D. B., Plant Physiology 2nd Edit., D. Van Nostrand Co., Inc. (1952).
- 3) Hashimoto, T., Sci. Pap. Coll. Gen. Educ. Univ. Tokyo **9**: 235 (1959).
- 4) Sass, J. E., Botanical Microtechnique 3rd Edit., The Iowa State College Press, Ames (1958).
- 5) DeRopp, R. S., Plant Physiol. **31**: 253 (1956).
- 6) Miller, C. O., *ibid.* **31**: 318 (1956).
- 7) Kuraishi, S., and Okumura, F. S., Bot. Mag. Tokyo **70**: 86 (1957).
- 8) Scott, R. H., Jr., and Liverman, J. L., Plant Physiol. **31**: 321 (1956).
- 9) Kuraishi, S., Sci. Pap. Coll. Gen. Educ. Univ. Tokyo **9**: 67 (1959).
- 10) Katsumi, M., Abstracts of Papers presented at the 24th Annual Meeting of the Botanical Society of Japan, 30 (in Jap.).
- 11) Jablonski, J. R., and Skoog, F., Physiol. Plantarum **7**: 16 (1954).
- 12) Naylor, J., Sander, G., and Skoog, F., *ibid.* **7**: 25 (1954).
- 13) Miller, C. O., Skoog, F., von Saltza, M. H., and Strong, F. M., Jour. Amer. Chem. Soc. **77**: 1392 (1955).
- 14) —, —, Okumura, F. S., —, and —, *ibid.* **78**: 1375 (1956).
- 15) Skoog, F., and Miller, C. O., Soc. Exptl. Biol. Symposium, Biological Action of Growth Substances, 11th Symposium, 118 (1957).
- 16) Das, N. K., Patau, K., and Skoog, F., Physiol. Plantarum **9**: 640 (1956).
- 17) Esau, K., Plant Anatomy, John Wiley and Sons, Inc., New York (1953).
- 18) Ball, E., Amer. Journ. Bot. **36**: 440 (1949).
- 19)

Popp, H. W., Bot. Gaz. **82**: 306 (1926). 20) Hashimoto, T., unpublished data. 21) Liverman, J. L., and Bonner, J., Proc. Natl. Acad. Sci., U.S. **39**: 905 (1953). 22) Schrank, A. R., Plant Physiol. **32**, suppl. xlix (1957). 23) Hillman, W. S., Science **126**: 165 (1957).

摘 要

橋本徹: エンドウの茎の1次肥大生長におけるインドール酢酸とカイネチンの相互作用

茎の伸長生長がオーキシンによって促進されることは周知の事実である。また形成層の活動による茎の二次肥大生長もオーキシンにより調節されていることがわかっている。しかし形成層による細胞の増殖を伴わない1次肥大生長を促進する物質はこれまで知られていなかった。私は野外で育てたアラスカエンドウの茎や葉柄の切片を用い、その1次肥大生長が、IAAとカイネチンとの協同的作用によっていちじるしく促進されることを見出した。

カイネチンはIAAによる茎および葉柄の伸長生長を抑制するが、肥大生長は、逆にいちじるしく促進する。この促進は、顕微鏡観察によれば、茎の表皮、皮層、および髓の細胞の容積の増加によるものであって、細胞の数の増加によるものではない。

茎では光照射によって、伸長生長は抑制され、肥大生長は促進されることが、これまで知られていたが、カイネチンは茎の生長において光とよく似た作用を有することが見出された。(武蔵大学生物学教室)

Seasonal Changes in the Osmotic Value, Water Content and Solute Ratio of the Bud of Mulberry Tree*

by Kiyoshi KAWANO**, Rikio TUZII** and Isao HATAKEYAMA***

Received September 1, 1960

It has been shown repeatedly by many investigators¹⁻⁸⁾ that cold hardiness of plants increases as a season enters from autumn to winter, reaching the highest value during midwinter and then decreases when the plants are exposed to warm weather in spring. As stated in most of their papers the osmotic value of plant tissue is large in a season when cold hardiness is presumed to be large, so it is supposed that there exists a relationship between the two.

The osmotic value of a cell in a normal state is due not only to the solute, but also to the water content. Hatakeyama⁹⁾ revealed that the solute ratio (solute content) was a valuable aid in interpreting the osmotic value differences. The solute ratio was calculated from the osmotic value of expressed sap and the water content on a dry weight basis, i.e., "solute ratio" = $P_{20} \cdot [\text{H}_2\text{O}] / 24.05$ where "solute ratio" = number of gram molecular weights (mols) of solute per kilogram of dry tissue, P_{20} = osmotic value in atmospheres at 20°, $[\text{H}_2\text{O}]$ = grams of water per gram of dry tissue, and 24.05 = the osmotic value of a mol of undissociated ideal solute in 1 kilogram of water at 20°. He¹⁾ also revealed in the study of the relation between freezing point and cold hardiness of plant tissue that the seasonal change in the freezing point of living tissue being an indicator of cold hardiness is considered to be due to variations in the colloiddally bound water in living state and in the solute ratio, but that of dead tissue by freezing is due mainly to the change in the solute ratio.

The authors²⁾ observed similarly in a previous paper that the freezing point of the living tissue in the mulberry bud was lower in cold season and its seasonal change depended on not only that of the freezing point of the dead tissue, but also that of colloiddally bound water in living state. But in that paper the details on the freezing point of the dead tissue by freezing were omitted out of space consideration, so those shall be discussed in this paper.

Material and Method

As material, the bud of *Akagi*, a cultivated race of *Morus bombycis* Koidz. in Mt. Hiei (600 m. above the sea-level) was used. In order to avoid variations as much as possible in values of measurement which might be influenced by such environmental factors as sunlight and rain, the material was collected early in the morning of a day next to a fine day.

A fine thermojunction was inserted in the bud and held in an air-space in a glass cylinder of 2 cm. diameter, soaked in the freezing mixture of -15°, until the bud temperature fell after freezing. The freezing point of the selfsame bud was measured repeatedly after thawing it. As the freezing was repeated, the freezing point rose

* Supported by a Grant in Aid for Scientific Research of the Ministry of Education.

** Moriological Institute, Faculty of Textile Fibers, Kyoto University of Industrial Arts and Textile Fibers, Kyoto, Japan.

*** Botanical Department, Faculty of Science, Kyoto University, Kyoto, Japan.

up, to become constant after the second to twelfth freezing when the tissue had died of freezing²).

This successive rise of freezing point will be due to liberation of colloiddally bound water in living cells^{1,2}). The highest freezing point of the dead tissue after the repeated freezing was still 0.1° lower than that of the brei of the selfsame bud, giving the same value as the expressed sap. This rise is considered to be due to liberation of the water imbibed or bound in cell wall, protoplasm and others after the sudden change of the micellar structure of the tissue¹).

Therefore, correction for the supercooling of the dead tissue and subtraction of 0.1° from the freezing-point lowering of it were done¹). Conversion of the freezing-point lowering to osmotic values at 0° was accomplished by Lewis' formula $P_0 = 12.06\Delta - 0.021\Delta^2$, where Δ = freezing-point depression. The value obtained was corrected for change in osmotic pressure from 0° to 20° by the equation $P_{20} = P_0 \cdot 293/273$. From this osmotic value and the water content of the selfsame bud, the solute ratio was calculated³).

Results

The results are shown in graphs (Fig. 1). The buds at this habitat unfolded in the middle of May. The new lateral buds were somewhat smaller in size than the old ones in spring. The osmotic value of new buds was low, while the water content was very large. And the solute ratio was relatively high. With the lapse of time after the budding the water content decreased and beyond that degree the osmotic value increased because of the increase of the solute ratio. From July to September, not only the water content but also the solute ratio decreased, so the osmotic value increased slightly.

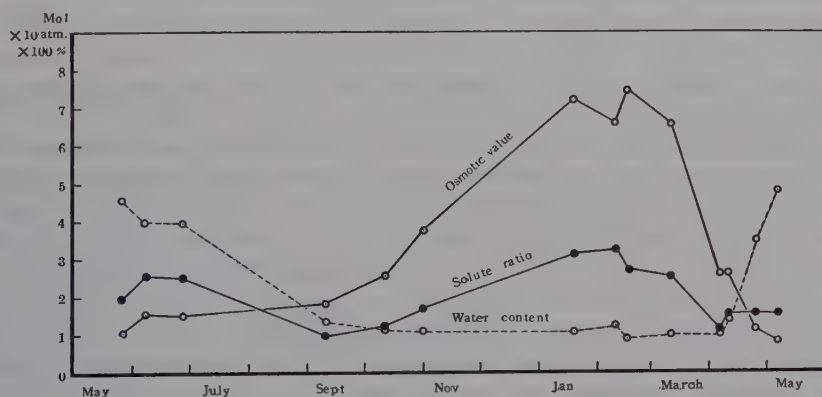


Fig. 1. Seasonal changes in the osmotic value, water content and solute ratio of mulberry buds.

From October to the beginning of April the water content remained almost constant, while the solute ratio increased from autumn to winter, reaching to its maximum in mid-winter and then decreased rapidly towards spring. Therefore, the osmotic value changed in the same way as the latter.

From the middle of April to that of May the water content increased rapidly, but the solute ratio remained almost constant except the slight rise in the beginning of April. So the osmotic value dropped to its minimum as rapid as in March. And soon after the experiment, the bud began to unfold.

Generally speaking, from May to September the change of the osmotic value is considered to be due to those of the water content and of the solute ratio, while from September to April of the next year it is due only to the change of the solute ratio and from April to May only to that of the water content.

Discussion

Such solutes as soluble saccharides, electrolytes and others and water content are playing a weighty part in changes of osmotic pressure of plant tissues.

Jeremias¹⁰⁾ revealed that in leaves of *Hedera helix* sucrose was seen in all seasons and showed its maximum in late winter when also galactose, raffinose and stachyose appearing in winter only reached to their maxima. Henze¹¹⁾ revealed that in barks of fruit-trees free pentose showed its maximum in November, while free hexose in February, but all pentoses containing bound ones showed more close correlation with frost hardness than all hexoses.

Parkers^{12,5)} observed the similar increase in sucrose, glucose, fructose, stachyose and especially raffinose of tree barks and leaves, and revealed⁶⁾ that raffinose increased in autumn from undetectable amount to about 1.5% of the fresh weight of white pine leaves in winter and was closely associated with hardness increase. The raffinose decline in spring was relatively steep, as was the nearly concomitant change in hardness.

Taguchi and Miyazaki¹³⁾ observed that the seasonal change in solute concentration in new shoots of fruit trees was due considerably to the change of electrolytes in the growing season and to that of non-electrolytes in winter. Also in the shoot of *Quercus* tree grown for rearing wild silkworms, Yamazaki *et al.*¹⁴⁾ observed that the content of reducing sugars increased greatly in winter and decreased in spring.

Using mulberry leaves Iwanari¹⁵⁾ observed that copper content in growing leaves in May was about twice as much as in full grown ones in October. Kashiwada¹⁶⁻¹⁸⁾ revealed that in mulberry shoots sucrose was seen all the year round, being more in August-September and March-April, and less in October-November. Glucose had a tendency to be constant throughout the year. Fructose was less in August, more in October-November and disappeared in March. In November, maltose, raffinose and stachyose appeared and their contents in winter were considered to be related to cold hardness. He observed raffinose and stachyose also in mulberry buds in winter.

Using the bark of mulberry twigs at Sapporo, Sakai^{7,8,19)} stated that stachyose first appeared in mid-October (ten days before the defoliation), then increased in the colder months, but it showed a marked decline in April and was not detectable in the latter part of the month (ten days before the budding). There was a marked increase in stachyose, raffinose, pentose as well as sucrose from autumn to winter, but no such increase in other sugars. The content of sucrose amounted in summer to 67% and in winter to 80% of the total sugar content.

In consideration of the premises, the osmotic value of each solute component in tissue is hard to understand, but the change of the solute ratio from May to September may be due considerably to that of electrolytes and from October to March to that of non-electrolytes. It may be able to say that September-October and April are the physiological turning-point of mulberry buds in Mt. Hiei because those months are not only the beginning and the end one of constant water content but also the time of the change of solute components.

The high osmotic value due to the high solute ratio as well as the large amount

of colloiddally bound water in living state²) is very favorable to cold hardness in winter, while the low osmotic value due to the large water content as well as the small amount of colloiddally bound water is a condition susceptible of frost damage in April-May.

Summary

The change of the osmotic value of mulberry buds is considered to be due to those of the water content and of the solute ratio from May to September. The water content being constant from September to April of the next year, the change of the osmotic value is due only to the change of the solute ratio, while the solute ratio being constant from April to May, the change of the osmotic value is due only to the change of the water content. It may be able to say that September-October and April are the physiological turning-point of mulberry buds in Mt. Hiei.

References

- 1) Hatakeyama, I., *Physiol. and Ecol. (Kyoto)* **7**: 89 (1957). 2) Kawano, K., Tuzii, R., and Hatakeyama, I., *Bull. Fac. Text. Fib. Kyoto Univ. of Industrial Arts and Textile Fibers* **3**: 32 (1960). 3) Levitt, J., *The Hardiness of Plants*. New York, p. 48, p. 73 (1956). 4) Parker, J., *Ecology* **36**: 377 (1955). 5) —, *Forest Science* **5**: 56 (1959). 6) —, *Bot. Gaz.* **121**: 46 (1959). 7) Sakai, A., *Low Temperature Science, Ser. B* **15**: 17 (1957). 8) —, *ibid.* **16**: 23 (1958). 9) Hatakeyama, I., *Physiol. and Ecol. (Kyoto)* **1**: 15 (1947). 10) Jeremias, K., *Planta* **52**: 195 (1958). 11) Henze, J., *Zeitschr. f. Bot.* **47**: 42 (1959). 12) Parker, J., *Naturwiss.* **45**: 139 (1958). 13) Taguchi, R., and Miyazaki, Y., *Agric. and Hortic. (Tokyo)* **28**: 522 (1953). 14) Yamazaki, H., Nishimura, K., and Taguchi, R., *J. Sericul. Sci. Japan* **25**: 71 (1956). 15) Iwanari, Y., *ibid.* **27**: 409 (1958). 16) Kashiwada, Y., *ibid.* **23**: 325 (1954). 17) —, *ibid.* **24**: 76 (1955). 18) —, *ibid.* **24**: 300 (1955). 19) Sakai, A., *J. Jap. Forestry Society* **42**: 97 (1960).

摘 要

河野清・辻井理貴雄・畠山伊佐男： クワの芽の浸透価，含水量および溶質比の季節的变化

クワの芽の連続凍結実験において，その氷点を熱電対を用いて測定した結果，生組織の氷点の季節的变化は生体膠質結合水と死組織氷点との季節的变化によることを前報²)で明らかにした。

死組織氷点から計算された浸透価の季節的变化は，5月から9月までは，含水量と溶質比との両方の変化によるものと考えられる。しかし，9月から翌春の4月までは含水量が殆んど一定であるから，浸透価の季節的变化は溶質比の変化にのみ依存し，一方4月から5月の芽の展開までは溶質比が一定であるから，含水量の変化にのみ依存する。

5月から9月までの溶質の変化には電解質が，10月から3月までのそれには非電解質が多く関与しているらしく，9～10月と4月は，含水量一定期間の前後ということからも，クワの芽の生理的転向点と考えられる。（京都工芸繊維大学繊維学部・京都大学理学部植物学教室）

Studies on Graft Hybrids of *Capsicum annuum* L.

I. Variation in Fruit Shape Caused by Grafting and the Effects in the First and Second Seed Generations*

by Noboru YAGISHITA**

Received September 6, 1960

Soviet authors have acknowledged the possibility that the change in life conditions induces change in some heritable characteristics of the organism. A number of Japanese biologists have seriously discussed on this problem, and some positive experimental data have been presented by Y. Sinoto¹⁾, J. Kasahara *et al.*²⁾, K. Hazama³⁾ and others.

Since 1954, the present author has been engaged in the studies on graft hybrids between two varieties of *Capsicum annuum* L. in the experimental field and greenhouse of the Faculty of Textile Industry of Tokyo University of Agriculture and Technology. These experiments have been made to examine whether any variation is induced or not by intervarietal grafting, and whether the variation, if any, is transmitted to the progeny or not.

Materials and Methods

Two varieties of the red pepper—*Capsicum annuum* L. var. *fasciculatum* Irish and var. *grossum* Sendt.—were used as materials for grafting; the former (called Yatsubusa) bears elongated cuspidal fruits in cluster (Fig. 1a), and the latter (Spanish Paprika) bears a single large bell-formed vallecule fruit on each node (Fig. 1b).

The seedlings used for grafting were of a pure line which had been preserved for generations at T. Sakata & Co. Each of the experimental plants was cultivated in a flowerpot. The seedlings bearing each 12-15 leaves were treated according to the method of cleft-grafting. The point of grafting was tightly bound with woolen yarn. In order to facilitate the tissue connection, grafted plant was covered



Fig. 1. Fruit shape of two varieties of *Capsicum annuum* L. used for grafting: a. Yatsubusa, b. Spanish Paprika.

with a beaker, kept in a shade in a greenhouse and watered enough. In general, 7-10 days were required for the stable connection between scion and stock, and then

* Reported at the 31st General Meeting of the Genetics Society of Japan (1959).

** Biological Laboratory, Faculty of General Education, Tokyo University of Agriculture and Technology, Fuchu, Tokyo, Japan.

the bandage was removed.

Yatsubusa was used for the scion as well as for the stock. In the case of the scion, a shoot of Yatsubusa with 4 to 5 leaves was grafted on the stock of Spanish Paprika, which was cut at a point on the 8th-10th internode from the bottom (Fig. 2a). In the case of the stock, a shoot of Spanish Paprika with 7-9 leaves was grafted on the stock of Yatsubusa which was cut on the 9th or 10th internode from the bottom (Fig. 2b). In both cases, some of the new leaves formed on Yatsubusa were picked off as soon as they came out, so as to avoid too much assimilation during the growing period.

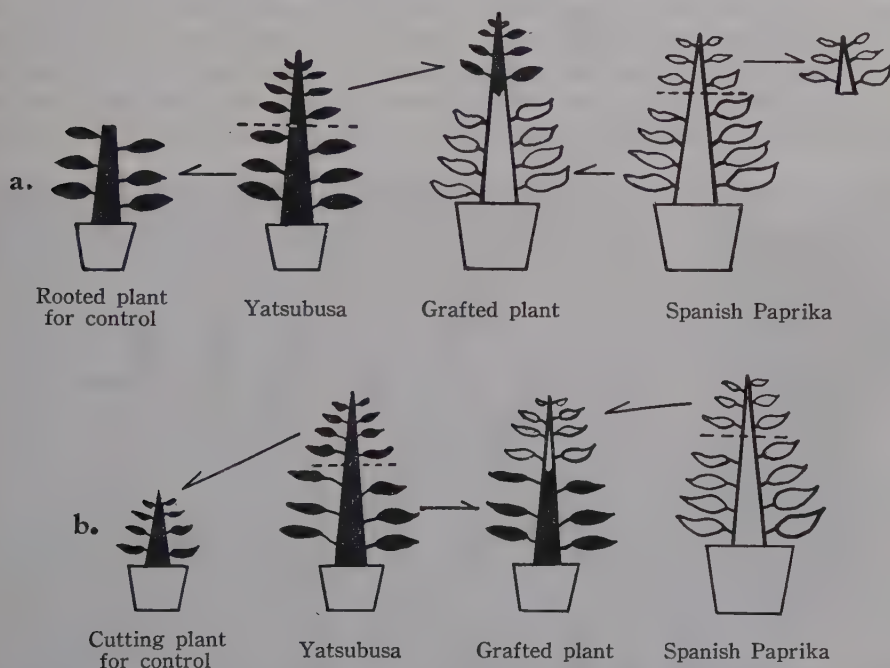


Fig. 2. Figures illustrating the methods of grafting and controlling: a. The scion of Yatsubusa was grafted on the stock of Spanish Paprika and the remaining rooted part of Yatsubusa was prereserved as control. b. The shoot of Spanish Paprika was grafted on the stock of Yatsubusa and the shoot of Yatsubusa was prereserved by cutting as the control.

Precaution was also made using the following plants as control: in 1954, the rooted plants of Yatsubusa, from which the scions had been cut, were prereserved (Fig. 2a), whilst the tops of the plants were cultivated by cutting (Fig. 2b). In 1955, the plants, which were raised from the seeds obtained by self-pollination of the control plants in 1954, were cultured; in 1956, the progeny of the second seed generation were used.

In order to avoid cross pollination, each flower of the grafted Yatsubusa was covered with a parchment-paper, but this treatment gave a deleterious effect on fruiting. Therefore, the grafted plants were grown in a greenhouse and the artificial pollination was made early in the morning. The control plants were also treated in a similar manner, but in another greenhouse. The flowers of Spanish Paprika,

which was used as stock or scion, were taken off at their stage of flower-bud.

In the present paper, the following abbreviations were used: GY_0 =grafted plants of Yatsubusa; GY_1 =the first and GY_2 =the second selfed generation of the GY_0 ; Y_0 =control plants for GY_0 ; Y_1 =those for GY_1 and Y_2 for GY_2 .

Results

(I) Results obtained by grafting on Yatsubusa-plant (GY_0):

In the grafting tried on June 5th, 1954, 14 out of 20 plants had successfully grown.

Some variations could be observed in the fruit shape of GY_0 . One fruit of a stock-plant (GY_0 -3) showed a clear-cut character of Spanish Paprika as shown in Fig. 3. This fruit is designated as GY_0 -311. Some of the other fruits of GY_0 -3 were round or sunken at the fruit top (Figs. 4 and 5a). These transformed fruits were found on a single plant together with standard ones characteristic for Yatsubusa. On a scion-plant (GY_0 -13), two transformed fruits were also found. Such a trans-













Fig. 3. The most conspicuous transformed fruit (GY_0 -311) of the GY_0 -3 plant produced by grafting with Spanish Paprika.

Fig. 4. Appearance of the GY_0 -3 plant bearing transformed fruits (shown by arrows) and standard ones.

Fig. 5. a. All fruits of the GY_0 -3 plant. b. All fruits of Y_0 -3, cutting control plant for GY_0 -3.

Fig. 6. Appearance of the Y_0 -3 plant.

Table 1. Variation in the fruit shape of the grafted plants of Yatsubusa; GY₀ (1954).

Grafted plants								Control plants							
Plant No. in GY ₀	Fruit types and No. of fruits						Used as	Plant No. in Y ₀	Fruit types and No. of fruits						Used as
	A	B	C	D	E	F			A	B	C	D	E	F	
						Abnormal								Abnormal	
3	—	4	1	3	3	—	ST*	3	—	7	—	—	—	—	C**
4	—	10	2	1	—	—	ST	4	—	—	—	—	—	—	C
5	—	10	3	—	—	—	ST	5	—	4	—	—	—	—	C
6	—	6	—	—	—	—	ST	6	—	5	—	—	—	—	C
7	—	5	—	—	—	—	ST	7	—	3	—	—	—	—	C
11	3	5	7	—	—	—	SC	11	—	11	—	—	—	—	R
12	—	6	1	—	—	—	SC	12	—	17	—	—	—	—	R
13	—	10	—	—	2	—	SC	13	—	7	—	—	—	—	R
14	—	15	—	—	—	1	SC	14	—	16	—	—	—	—	R
15	—	1	5	—	—	1	SC	15	—	11	—	—	—	—	R
16	12	1	—	—	—	—	SC	16	4	11	—	—	—	—	R
17	—	4	1	—	—	—	SC	17	—	11	—	—	—	—	R
19	—	11	2	—	—	—	SC	19	—	14	—	—	—	—	R
20	—	13	—	—	—	—	SC	20	—	15	—	—	—	—	R
Total %	15 10.1	101 67.8	22 14.9	4 2.6	5 3.3	2 1.3		Total %	4 2.9	132 97.1	0 0	0 0	0 0	0 0	

* ST: Stock. ** C: Cutting plant.
SC: Scion. R: Rooted plant.

formation in fruit shape was never observed on the control grown by cutting, *i.e.*, Y₀-3 for GY₀-3, Y₀-13 for GY₀-13, and so on (Table 1 and Figs. 5b and 6). In the next year it was found that all the fruits of hibernated Y₀-3 plant were also of a standard shape.








(II) Results obtained in the First Selfed Generation of the Grafted Plant (GY₁):

Out of the fruits of GY₀-3, four fruits of different shapes were selected for sowing: (1) conspicuously transformed, GY₀-311 (2) a little transformed, GY₀-312 (3) not transformed, GY₀-321 (4) sunken at the top, GY₀-331. A portion of the seeds in each group were sown separately. The seeds obtained from Y₀-3 were sown as the control for GY₁. Besides, cutting plant Y₀-3 which had hibernated in a greenhouse was used again as the control.

All the seeds were sown in Petri-dishes on April 17th, and the seedlings were transplanted to the nurseries of wooden box in a greenhouse, and afterwards, each plant with 12-15 leaves was transplanted to a flower-pot.

The results in the first selfed generation from GY₀-3 and Y₀-3 are given in Table 2. The range of variation in GY₁ was found to be greater than that of GY₀. The

Table 2. Results in the first selfed generation of the grafted plant; GY₁ (1955).

		First selfed generation of the grafted plant						
		Fruit types and No. of fruits						
Fruit No. in GY ₀	No. of plants	A	B	C	D	E	F	G
								
311	11	—	47	4	—	—	2	1
312	4	—	11	3	4	1	—	1
321	10	—	52	—	—	—	—	—
331	7	—	21	2	1	6	—	4
Total	32	0	131	9	5	7	2	6
%		0	81.9	5.6	3.1	4.4	1.2	3.8
		Control plants						
Y ₀ -3*	1	—	7	—	—	—	—	—
Y ₁ -3	1	—	6	—	—	—	—	—

* Y₀-3: Hibernated plant.Y₁-3: Selfed progeny.

transformed fruits are classified as shown in Table 2. One fruit from Y₀-311 family, had the same valleculeae as GY₀-311 fruit in the previous generation (Fig. 7). This fruit was designated as GY₁-311-1. One fruit out of GY₀-312 family and four out of GY₀-331 family showed no cuspidation. They were round or sunken at the fruit-top (Table 2).

The appearance of the transformed fruits in this generation was similar to that in GY₀. These transformed fruits were found among the standard fruits (Fig. 8). The frequency of occurrence of the transformed fruits was 3.8 per cent. The progeny from GY₀-321 fruit which had not been transformed in GY₀, showed no transformation in this generation, too (Table 2).

The Y₁-3 plant, the selfed progeny of Y₀-3 and the hibernated Y₀-3 plant had the fruits only of the standard shape (Figs. 9 and 10).

(III) Results obtained in the Second Selfed Generation (GY₂):

In this generation, two parent plants were chosen out of twelve plants of GY₁-311 family in 1955 and one fruit was taken from each of them. And seeds therefrom were sown for GY₂ test in 1956. These two fruits were designated as GY₁-311-2 (C) and GY₁-311-6 (F). On the other hand, two fruits, GY₁-331-3 (B) and GY₁-331-7 (G) from two of the GY₁-331 family were selected and sown. The letters in parentheses correspond to those of the classification of fruit shapes in Table 2. As the seeds of the GY₁-311-1 fruit (G) remained immature, the seeds of GY₁-311-2 obtained from the same plant were sown instead of them. Selfed progeny from Y₁-3, namely, Y₂-3 plants were used as the control in this year. The same methods of cultivation as those in 1955 were adopted. The date of sowing was April 20th.

In this generation, four out of seventeen plants bore five fruits with valleculeae,



Fig. 7. The most transformed fruit (GY₁-311-1) appeared in the first selfed generation from GY₀-311.

Fig. 8. Appearance of the plant bearing the GY₁-311-1 fruit.








Fig. 9. Y₁-3 plant, first progeny of the control.

Fig. 10. Control plant, hibernated Y₀-3.

one of the diagnostic characters of Spanish Paprika. The details of the results are shown in Table 3. Frequency of occurrence of these transformed fruits was 2.4 per cent. Comparison between GY₁ and GY₂ as regards frequency of occurrence of the transformed fruits is shown in Fig. 11. One fruit from GY₁-331-3 family showed the most conspicuous characters of Spanish Paprika. The fruit became bigger and shorter, and it had deep decussate vallecule on fruit top (Fig. 12). This and the other transformed fruits were found among the standard ones in one cluster (Fig. 13). It is noteworthy that a remarkable difference in shape was observed between this fruit and the mother GY₁-331-3 fruit (Fig. 14).

Although there was some aberration in fruit shape of the control plants, Y₂-3

Table 3. Results in the second selfed generation of the grafted plant; GY₂ (1956).

Second generation of the grafted plant								
Fruit types and No. of fruits								
Fruit No. in GY ₁	No. of plants	A	B	C	D	E	F	G
								
311-2	6	39	4	3	3	4	16	1
311-6	3	24	4	—	6	—	1	2
331-3	5	17	31	1	1	—	—	1
331-7	3	31	12	4	—	—	—	1
Total	17	111	51	8	10	4	17	5
%		53.9	24.8	3.9	4.9	1.9	8.2	2.4
Second generation of the control plant								
Y ₂ -3	10	66	3	1	2	—	—	—
%		91.7	4.2	1.4	2.7	0	0	0

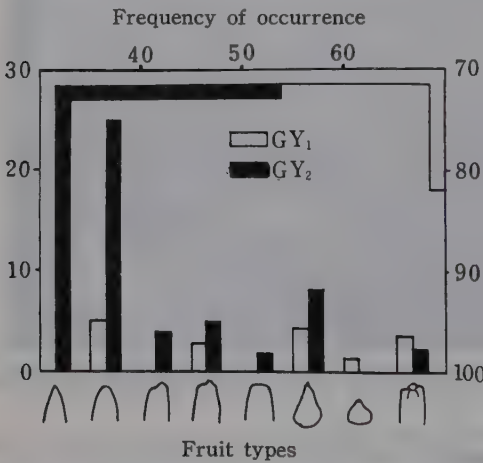


Fig. 11. Comparison between GY₁ and GY₂ in frequency of occurrence of the transformed fruit.

(Fig. 15). The F₁ plants bore a single fruit upside down on each node. This result was quite different from that of the grafting between the same two varieties.

As shown in Table 4, it was observed that the results of grafting, crossing, and control experiments were distinguishable from each other. For further evaluation of the effect of grafting, the author has conducted the experiments by successive graftings for three generations. The results will be reported elsewhere. Conclusive discussion will also be described therein.

(Table 3), remarkable transformation such as observed in GY₀, GY₁ and GY₂ did not occur in Y₂-3 plant.

(IV) Results obtained from the Cross between Yatsubusa and Spanish Paprika:

In order to see whether or not the variation in fruit shape by grafting is due to natural crossing, the artificial crossing, Yatsubusa × Spanish Paprika was tried in 1956. The mother plant belonged to Y₂-3 plants. The seeds of a fruit obtained by this cross were sown for the F₁ test in 1957. The results of the F₁ test were as follows; all F₁ plants produced the uniform fruits which were much bigger than those of Yatsubusa

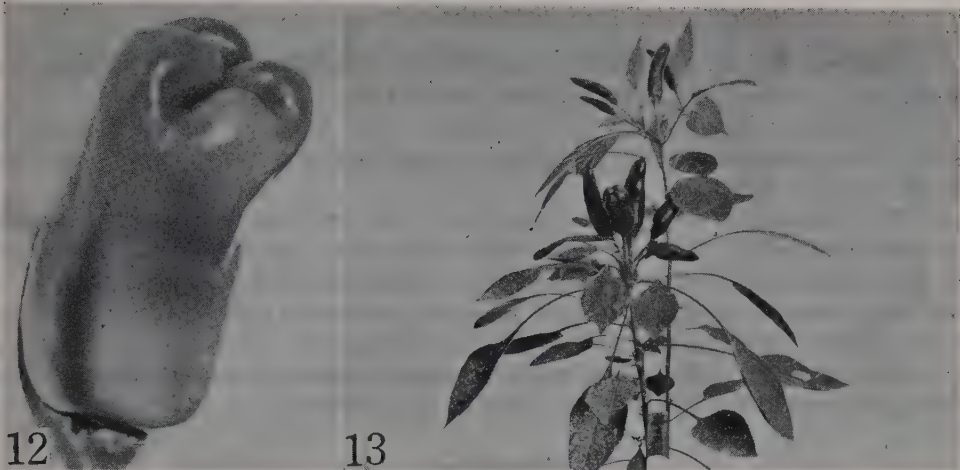


Fig. 12. A transformed fruit appeared in the GY₁-331-3 family.
Fig. 13. The transformed fruit coexisting with standard fruits of Yatsubusa.



Fig. 14. The mother fruit which produced the transformed fruit (left) and other fruits on the same plant.
Fig. 15. a. Fruit born in F₁ of the crossing, Yatsubusa×Spanish Paprika. b. Mother fruit in this crossing, Yatsubusa.

Table 4. Some diagnostic characters of mother plants, F₁ plant and grafted plant.

	Habit of branching	Habit of fruiting	Fruit shape
Yatsubusa	weak	bearing several fruits upright in a cluster	elongated, cuspidal
Spanish Paprika	strong	bearing a single fruit upside down on each node	large bell-shaped, having valliculae on the fruit top
Yatsubusa × Spanish Paprika	same as Spanish Paprika	same as Spanish Paprika	same as the fruit shape of Yatsubusa but much bigger
Yatsubusa + Spanish Paprika	same as Yatsubusa	same as Yatsubusa	elongated but multiform on the fruit top; (a) valliculate, (b) sunken, (c) round, (d) cuspidal

Summary

1. Experiments on grafting between *Capsicum annuum* L. var. *fasciculatum* Irish and var. *grossum* Sendt. were carried out in 1954.

2. Distinctive transformation in fruit shape, which might be due to an effect of grafting, was observed in two grafted plants, and the transformed fruits coexisted with the standard ones.

3. The variation in fruit shape appearing in the first and second selfed generations was investigated. It was found that the diagnostic characters of transformed fruit were transmitted to the progeny of the following two generations.

4. Such variation as found in fruit shape of the grafted plants and their progeny was not observed on the control plants for three successive generations.

5. The variation in F_1 plants obtained by sexual crossing between the same two varieties was essentially different from that of the grafted plants and the progeny.

References

- 1) Sinoto, Y., Kagaku, **25**: 602 (1955). 2) Kasahara, J., Nakamura, T., and Yoneyama, Y., Journ. Agr. Iwate Univ. **2**: 149 (1955). 3) Hazama, K., Biological Science (Tokyo), **10**: 172 (1958).

摘 要

柳下登： トウガラシの接木雑種に関する研究 I. 接木による果形の変異と第1および第2の自家受精世代におよぼす影響

トウガラシ (*Capsicum annuum* L.) の2品種間の接木試験を 1954 年におこなった。

接木植物の果形に、接木の影響とみられる変異があらわれた。この変異は第1および第2の自家受精世代にも伝えられた。

3代にわたる対照植物には、接木植物とその子孫にみられたような変異は、観察されなかった。

接木実験に用いたものと同じ材料について、それぞれ有性交配がおこなわれた。その F_1 植物での果形の変異の状態は、接木植物およびその子孫にみられた変異とは全くちがっていた。(東京農工大学一般教育部生物学研究室)

Studies on the Germination of Grass Pollen II Germination Capacity of Pollen in Relation to the Maturity of Pollen and Stigma

by Kotaro WATANABE*

Received September 9, 1960

In a previous paper¹⁾, the author reported that grass pollen grains exude a liquid before germination on their own stigmas, and those which exude no liquid are incapable of germination. To study more in detail the relationships between germination and liquid exudation, the germination ratio and behaviour of pollen on the stigma were examined, especially in relation to the maturity of both pollen and stigma.

Materials and methods

Main materials used were barley (*Hordeum vulgare*), common wheat (*Triticum vulgare*), Einkorn wheat (*Triticum monococcum*), rye (*Secale cereale*), maize (*Zea Mays*) and rice (*Oryza sativa*).

Some fresh stigmas were placed on a slide-glass and pollinated with pollen from splitting anthers. After placing the preparation for 1–2 hours in Petri-dish with or without a sheet of wet filter paper, the materials were stained with aceto-carmin, and all the grains which had adhered to the stigma were examined. In maize, the tips of stigmas, about 5–10 mm. in length, were usually observed. In the case of natural pollination, the florets which bloomed on the day or on the preceding day of experiment were chosen. The stigmas to which the pollen grains adhered in crowds were discarded.

The stigma hair, consisting of four rows of cells, has at first a form of a smooth column. Then each cell of the hair begins to project at the upper end and grows to maturity. Only the stages of immature stigma or stigma hair will be distinguished below as “smooth-” and “papillate-stage” (Fig. 1). The stigma does not uniformly develop over the full length, but the tip develops earlier than the base. In the course of development there appears the stigma which has the stigma hairs of the smooth-stage at the base and those of the papillate-stage at the tip. Such a stigma will be described below as “intermediate-stage”. In the long stigma (silk) of maize, the upper parts were at times used as the material of papillate-stage, and the lower parts as that of smooth-stage stigma. The process of the stigma development in *Zea* has been described by Weatherwax²⁾.

The developmental processes of grass pollen grains have already been studied in wheat³⁾, rye⁴⁾ and rice⁵⁾. In the pollen grains of common and Einkorn wheat, the present author observed that starch grains appear after the translocation of the two pollen nuclei to the neighbourhood of the germ pore. They increase once in number as the vacuole becomes smaller, and fill up the pollen grain with the complete disappearance of the vacuole. Before the anther dehiscence, the starch in many pollen grains decreases at the end opposite to the germ pore^{6,7)}. This phenomenon of starch decrease was recognized in all the species observed, i.e. in wheats, rye, maize and rice. It was especially remarkable in rye. In the following two types of grains,

* Laboratory of Applied Botany, Faculty of Agriculture, Kyoto University, Kyoto, Japan.

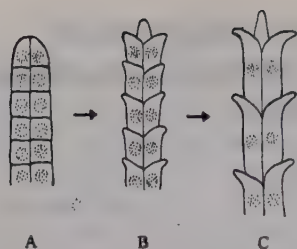


Fig. 1. Schema of the development of stigma hair

A-B: Immature

A: Smooth-stage hair

B: Papillate-stage hair

C: Mature

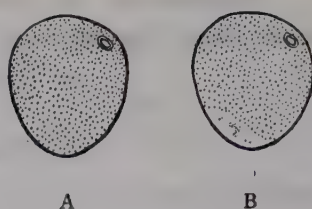


Fig. 2. Two types of pollen grains

A. Starch-stage grain

B. Sugar-stage grain

Starch grains are shown with the dots.

namely, those densely filled with starch grains and those in which starch is more or less decreased, will be provisionally called "starch-stage" and "sugar-stage" grains (Fig. 2), respectively. In general there exist no morphological differences between these two forms. The grain of the starch-stage is, however, stained dark red or reddish brown in colour with aceto-carmin, while that of the sugar-stage is stained light red or reddish orange with the same dye solution. From a dehiscent anther, in most cases, there appear pollen grains of these two stages.

Results

1. Germination percentage of pollen on mature stigma

There are tolerable fluctuations in the germination percentage of pollen on individual stigma. The present data show, however, that the pollens show 60–80 per cent germination on their own stigmas with both natural as well as artificial pollination (Table 1). The reason why the remaining, apparently mature grains do not germinate may lie both in the nature of stigma and in that of the pollen grain itself.

Table 1. Germination percentage of pollen on the mature stigma

Species	Art of pollination	No. of stigma invest.*	No. of grains Germ./Observed	Germination percentage
<i>Agropyrum ciliare</i>	natural	148	1480/2036	72.7
<i>Avena sativa</i>	natural	31	691/1037	66.6
<i>Hordeum vulgare</i> (cult. var.** Sangatsu-Mugi)	natural	49	1054/1713	61.5
	artificial	16	258/376	68.6
<i>Oryza sativa</i> (cult. var. Kyoto-Asahi)	natural	66	1654/2376	69.6
<i>Poa annua</i>	natural	101	1785/2623	68.1
<i>Secale cereale</i>	artificial	17	1084/1466	73.9
<i>Triticum vulgare</i> (cult. var. Norin No. 26)	natural	17	888/1390	63.9
	artificial	7	456/729	62.6
<i>Zea Mays</i> (flint corn)	natural	20	426/511	83.4
(pop corn)	natural	39	537/849	63.3

* Excepting *Zea*, one of the two branches of a stigma was counted as a unit. This is also the case with the other tables.

** cult. var.: cultivated variety.

2. Germination and maturity of pollen

Niethammer⁸⁾ tested the germination of pollen in various species by artificial media, and found that the group of pollen which germinated well contained much sugar, whereas another group showing no germination had a large quantity of starch, but not any detectable sugar. The similar starch-germination relationship is already found in *Cassia Fistula* (Tischler⁹⁾, "Befruchtungs-" and "Beköstigungspollen"*) and later by Sisa¹⁰⁾ in the pollen of loquat (*Eriobotrya japonica*).

As shown in Table 2, most of the grains of Einkorn wheat which did not germinate on the stigma belong to those of the starch-stage, i.e. those densely filled with

Table 2. Types of pollen grains not germinated on the mature stigma.
Triticum monococcum (Precocious strain).

Situation of floret on ear	No. of stigma examined	No. of pollen grains		Percentage of	
		Sugar-stage	Starch-stage	Sugar-stage	Starch-stage
Top	10	21	363	5.5	94.5
Middle	20	57	363	13.6	86.4

Table 3. Relations between pollen maturity and germination capacity. *Secale cereale*.

Stage of pollen grain	No. of mature stigma examined	No. of pollen grain (Germ./Observ.)	Germination Percentage
Starch stage	4	3/661	0.5
Starch-stage, containing a few sugar stage ones	8	92/831	11.1
Starch-stage, containing rather many sugar-stage ones	8	184/850	21.6
Sugar-stage,* containing some starch stage ones	4	260/604	43.0

* Pollen grains from florets flowered on the ear cut before natural flowering.

starch grains. It is also evident, as shown with rye pollen in Table 3, that the pollen grains belonging to the starch-stage germinate with difficulty. Here, the starch-germination relationship is also recognized.

Most of the starch-stage grains exude no liquid on the stigma. They shrink sooner or later. Even when they germinate, there develop poor pollen tubes in most cases. More immature grains have no germination capacity.

* By adding diastase, Tischler found that the "Beköstigungspollen" secured the ability to produce the pollen tube. He also found the pollen of many other tropical plants passed the starch stage.

A few remarks may be mentioned here. In rye and Einkorn wheat, even the sugar-stage grains were rather difficult to germinate, as they were obtained from anthers before natural dehiscence by cutting the ear before flowering (Table 3) or taking out the anther from a closed floret. The anthers in one and the same floret do not necessarily develop with the same tempo. In one case in Einkorn wheat, two of the three anthers in a floret contained almost wholly the starch-stage and more immature grains, whereas the other had a great majority of sugar-stage grains.

3. Behaviour of pollen grains on immature stigma

As shown in Table 4, the germination percentage of pollen grains on the immature stigma is not so good as on the mature stigma, with the exception of *Zea Mays*. Generally, the germination of pollen grains becomes better as the stigma develops.

Tablbe 4. Germination of mature pollen grains on immature stigma.

Species	Humidity condition	Stage of stigma	No. of stigma	No. of grains Germ./observ.	Germ. percent.
<i>Hordeum vulgare</i> C.v. ¹⁾ "Trebei I"	Moist chamber	S & P ²⁾	80	366/2049	17.9
		(M)	(8)	(343/519)	(66.1)
	C.v. "Coast I"	S & P (M)	54 (30)	306/959 (1439/1803)	31.9 (79.8)
<i>Oryza sativa</i> C.v. "Kyoto Asahi"	Moist chamber	P (M)	38 (8)	247/1091 (246/410)	22.6 (60.0)
	In the room	P (M)	25 (10)	100/596 (230/433)	16.8 (53.1)
<i>Secale cereale</i>	Moist chamber	S	59	74/960	7.7
		I	8	35/234	15.0
		P (M)	4 (16)	39/141 (954/1648)	27.7 (57.9)
	In the room	S & I P (M)	58 22 (14)	321/1417 542/1433 (1452/2222)	22.7 37.8 (65.3)
<i>Triticum vulgare</i> C.v. "Akakawa-Aka"	In the room	S	10	44/277	15.9
		I	8	91/338	26.9
		P	32	606/1528	39.7
		(M)	(10)	(627/983)	(63.8)
<i>Triticum monococcum</i> var. <i>vulgare</i>	Moist chamber	S	4	0/92	0
		I	8	37/288	12.8
		P	12	99/479	20.7
		(M)	(4)	(422/611)	(69.1)
	In the room	S	16	47/381	12.3
		I	18	33/465	7.1
		P	10	107/446	24.0
		(M)	(12)	(1026/1431)	(71.7)
<i>Zea Mays</i> C.v. "Koshu"	In the room	S	55	1365/1957	69.7
		I	5	238/289	82.4
		P	30	382/444	86.0
		(M)	(45)	(1366/1908)	(71.6)

1) C.v.: Cultivated variety.

2) S, Smooth-stage; I, Intermediate-stage; P, Papillate-stage in the immature stigma; M, Mature-stage of stigma.

The behaviour of pollen on the immature stigma was compared with those on the mature stigma. An excised immature stigma and a mature one were placed on a slide-glass side by side, and simultaneously pollinated with pollen from one and the same anther. The preparation was immediately brought under the microscope.

On the immature stigma, the liquid exudation from the grain surface, the "wart-like form change"¹⁾, occurred generally later and proceeded more sluggishly than on the mature stigma; in *Secale*, the exudation began on the mature stigma 10–15 seconds after contact with the stigma cells, while on the immature stigma it began in most cases after about 40–45 seconds. The pollen grains showing the exudation were small in number on the immature stigma (and others shrunk sooner or later), whereas on the mature stigma it occurred in most of the grains. On the immature stigma, the amount of liquid oozing out between stigma cells and pollen grain was also smaller than that on the mature stigma, and many of the grains burst soon after the liquid exudation or just after producing the tube. From the latter effect, the germination ratio of pollen on the immature stigma became much smaller. As the stigma advanced to maturity, the frequency of the liquid exudation of pollen became larger and that of bursting smaller.

4. Growth of pollen tube on immature stigma

Usually the pollen tube emerging on the immature stigma grew very poorly. In *Secale* the tube, if elongated to some extent, did not enter the immature stigmatic tissue of the smooth-stage. Sometimes the tip of the tube swelled spherically and some of them burst thereafter. There also appeared pollen tubes thinner or thicker than normal. Often the tubes growing along the grain surface suddenly burst at the tip as soon as they came into contact with the stigma cells. In *Zea*, the same phenomenon was also often seen on the mature stigma: exceptionally the tube entered immature stigmatic tissue of the smooth-stage at the point where the tip of the stigma rachis divided into forks.

5. Occurrence of reducing sugar in pollen and stigma

With Fehling's solution, reducing sugar in the pollen grain and stigma were examined in Einkorn wheat. The preparation was heated to the boiling point and soon observed under the microscope.

Sugar content was greater in the pollen grain than in the stigma, but it was difficult to find any differences in the content between starch- and sugar-stage grains. As the stigma developed, the sugar content also increased. More precipitation of cuprous oxide was observed in the stigma rachis than in the stigma hair. In the immature, especially in the smooth- or intermediate-stage stigma hairs, they could scarcely be detected.

Considerations

From the results obtained above, the relation of the germination capacity of pollen to the maturity of both pollen and stigma may be summarized as follows (Table 5):

Table 5. Maturity of pollen and stigma in relation to the pollen germination

Pollen \ Stigma	Immature	Mature
	(No germination?)	Bad or no germination; Non or almost no liquid exudation; Shrinkage but no bursting
Immature		
Mature	Bad germination; Non or little liquid exudation; Shrinkage or bursting	Good germination; Remarkable liquid exudation; Some shrink or burst

It can be seen from the table that a liquid appears between pollen and stigma whenever the pollen grains are capable of germination. Good germination is, however, secured only when the mature pollen grains fall on the mature stigma. Pollen grains of various maturity stages are contained even in one and the same dehiscing anther, and the germination percentage on the mature stigma is influenced by the ratio of pollen grains of a certain developmental stage. The grain filled with starch produces the tube with difficulty. Most of it shrinks on the stigma, and never shows any remarkable liquid exudation.

On the mature stigma, most of the mature pollen grains exude liquid soon after coming into contact with the stigma cells, and then the tube emerges. The grains which do not exude the liquid do not germinate, but begin to shrink. On the immature stigma, many of the mature grains shrink sooner or later, and even when the grains exude a small quantity of liquid, many of them burst before or after the emerging of the pollen tube; the nature of the liquid on immature stigmas seems to be different from that on mature ones. As will be stated in detail in another paper, the stigma reaction^{11,12}), i.e. a rapid increase in the permeability of stigma cells immediately (within one minute or so) after pollination, occurs most conspicuously at the portion of the stigma hair where the pollen grain shows "wart-like form change" or visible liquid exudation. When the stigma reaction occurs, the liquid covers the surfaces of the stigma hair and the pollen grain, and, presumably, the liquid at that time is derived from both of the partners.

The exuded ('normal') liquid seems to be highly viscous¹). Besides moderate water supply¹³⁻¹⁵), some agents contained in it may stimulate the germination. In a cultivated variety of rice, for example, some amino acids (serine and alanine) are abundant in both pollen and pistil, and with the addition of these on the artificial medium the germination ratio of the pollen becomes larger¹⁶). A similar effect of amino acid contained in pollen and stigma is also recognized in maize¹⁷). The difference of sugar content between mature and immature stigma is also to be concerned in the germination.

In *Zea* the pollen grains germinate well on both mature and immature stigmas, but on the immature stigma many of the grains do not exude the liquid for a few minutes after contact with the stigma cells. This is also the case with other species. The pollen grains of *Zea*, however, can keep their turgid state on the immature stigmas and can germinate after a considerably long time, e.g. 30-40 minutes after pollination. The pollen of this species is known to be tolerable to a wide range of water supply for germination^{13,18}). The peculiarity for water absorption and osmoregulation of the pollen grains as well as the water condition of the stigma may, in

this case, have great influence upon the pollen germination.

Summary

1. On the stigma, the grass pollen shows about 60-80 per cent germination under natural as well as artificial pollination. The pollen grains, which are apparently mature but germinate with difficulty, are densely filled with starch grains, whereas in the pollen grains which are capable of germination the starch grains are decreased at the end opposite to the germ pore. More immature pollen has no germination capacity.

2. The germination on immature stigma is not so good as that on mature stigma. The percentage of germination increases as the stigma ripens. As an exception, the maize pollen can germinate well on both mature and immature stigma.

3. The liquid that oozes out between pollen grain and stigma cells before the pollen tube emerges seems to be derived from both of the partners, and also to stimulate the pollen germination with some agents contained in it.

Grateful acknowledgment is due to Prof. S. Imamura, and Assoc. Prof. M. Hamada of the laboratory, and to Lect. K. Kato of the Inst. of Botany, Faculty of Sci., Kyoto University, for their kind advice and criticism.

References

- 1) Watanabe, K., Bot. Mag. Tokyo **68**: 40 (1955).
- 2) Weatherwax, P., Bull. Torrey Bot. Club **44**: 483 (1917).
- 3) Kihara, H., Mem. Coll. Agr. Kyoto Imp. Univ. **41**: 1 (1937).
- 4) Takagi, F., Jap. Jour. Genetics **12**: 69 (1936) (in Jap.).
- 5) Kihara, H., and Hirayoshi, I., Agric. and Hort. **17**: 685 (1942) (in Jap. with Eng. Summary).
- 6) Percival, J., The wheat plant. London (1921).
- 7) Wodehouse, R.P., Pollen grains. New York/London (1935).
- 8) Niethammer, A., Biochem. Zeitschr. **249**: 412 (1932).
- 9) Tischler, G., Jahrb. f. wiss. Bot. **47**: 219 (1910).
- 10) Sisa, M., Jour. Hort. Assoc. Japan **4**: 141 (1933) (in Jap.).
- 11) Kato, K., Mem. Coll. Sci. Univ. Kyoto, Ser. B, **20**: 203 (1953).
- 12) Kato, K., and Watanabe, K., Bot. Mag. Tokyo **70**: 66 (1957).
- 13) Jost, L., Ber. dtsch. bot. Ges. **23**: 504 (1905).
- 14) Anthony, S., and Harlan, H. V., Jour. Agric. Res. **18**: 525 (1920).
- 15) Kubo, A., Jour. Sci. Hiroshima Univ., Ser., B, **7**: 103 (1956).
- 16) Sawada, Y., Bot. Mag. Tokyo **71**: 218 (1958). (in Jap. with Eng. Summary).
- 17) —, ibid. **73**: 252 (1960) (in Jap. with Eng. Summary).
- 18) Pfundt, M., Jahrb. f. wiss. Bot. **47**: 1 (1909).

摘 要

渡辺光太郎： 穀類花粉の発芽に関する研究 II 花粉および柱頭の熟度と発芽との関係

1. イネ科の花粉は一般に柱頭上で 60~80% の発芽率を示す。その柱頭接着直後、粒面から一種の液を滲出し、柱頭細胞との間に液の存在が明らかな花粉粒のみが発芽できる。

2. 発達の過程において花粉粒内には、いったんでんぶん粒が充満するが、葯裂開前に発芽孔と反対側の端部でその消失が起こる。でんぶん粒を充満する花粉粒は発芽能力が低く、それ以前の發育過程にあるものは発芽しない。裂開葯からの花粉が成熟柱頭上ですべて発芽しないのは、同一葯中ででんぶん含量の異なる花粉粒の混在に原因するところが多い。

3. 未熟柱頭上では成熟柱頭上よりも花粉発芽率は低い (トウモロコシは例外)。かつその程度は柱頭の未熟なほど著しい。未熟柱頭上では、1) 花粉粒の多くが液を出さず、そのままシワが寄り、2) 液を出す粒も滲出程度が弱く、花粉粒—柱頭細胞間に現われる液が少量であるにもかかわらず、花粉管の生ずる前後にしばしば破裂する。3) また管生長も異常なことが多い。

4. 受粉直後に起こる受粉部柱頭細胞の顕著な透過性増大 (柱頭反応) の事実とあわせ、発芽前に花粉—柱頭間に現われる液はこの両者、すなわち花粉粒と柱頭細胞の両者から由来したものであり、かつその中に花粉の発芽を促進または可能にする要素をふくむことを考察した。(京都大学農学部応用植物学研究室)

水生菌類の遊走子の日周的な垂直移動

鈴木 静 夫*

Shizuo SUZUKI: The Diurnal Migration of Zoospores of Aquatic Fungi in a Shallow Lake.

1860 年 6 月 8 日受付

運動力を持つ水生菌類の遊走子が、湖沼中で動物プランクトンに広く認められるような日周的な垂直移動を行なうか否かは興味ある問題である。水生菌類の遊走子は動物性プランクトンに比べて運動力が小さいので、この問題の解明には水深の浅い池沼が好つごうである。そこで著者は、水深が 0.5 m 前後にすぎない東京教育大学の構内にある占春池において、1955 年から 1956 年までの 1 年間、この点について観察を行なったので、その結果を報告する。

研究 方 法

採水は岸からやや離れた地点で、サイホンを利用して表層 (0 cm)、中層 (25 cm)、底層 (50 cm) の 3 層の水を採集し、同時に水温と溶存酸素量を測定した。遊走子の定量は前報と同じ方法によった¹⁾。

水生菌類の遊走子の垂直移動

水生菌類の遊走子は 2 本のぺん毛を有し、走化性を示し、特に溶存酸素量の良好な部分に集まることが知られている。Höhnk²⁾、Cotner³⁾ は水生菌類の遊走子が好気性で、懸滴水中において酸素の多い表層に集まることを報告している。このような事実が、池中でも存在するか否かを知るために、遊走子の分布と酸素の垂直分布との相関関係を追求した。

1. 春季および秋季

水生菌類の遊走子の垂直移動は、環境条件の類似している春季と秋季とではほぼ同様であるが、天候によって多少異なる。

晴天: 溶存酸素の垂直分布の時間的变化 (第 1 図) に伴って、水生菌類の遊走子の垂直分布も第 2 図に示すような日変化を示した。すなわち、午前 7

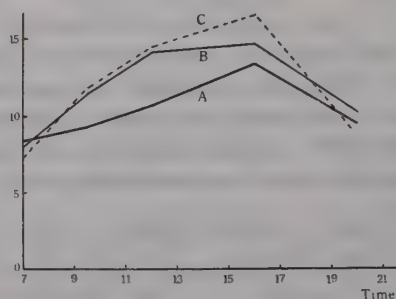


Fig. 1. Diurnal changes of amount of oxygen in clear day

A: surface layer
B: middle layer
C: bottom layer

—8 時頃には遊走子は酸素の最も多い表層に集まり、水底に行くに従って減少し、底層にはごく少数の遊走子が見られるにすぎない。時間がたつにつれて底層に酸素が増加するので、遊走子は底層にも分布するようになり、表層から水底まではほとんど一様な分布を示す。午後 4 時頃にはこの傾向が特に顕著で、遊走子は酸素の最も多い底層に多く見られるようになる。日没後 2—3 時間たつと、底層の酸素の減少に伴って、遊走子は酸素の多い表層と中層に集まる。

曇天: 1 日を通じてわずかではあるが常に表層に酸素が多いので、水生菌類の遊走子は表層に集まり、中層と底層には非常に少ないか、あるいは全く見られず、遊走子の垂直移動は認められない。特に表層でも水面に最も多く、水面より 5 cm 下の水には遊走子はほとんど見られないが、これは溶存酸素量の勾配によるものと考えられる。

雨天: 酸素の垂直分布は曇天の場合とだいたい同じであるが、水生菌類の遊走子は 1 日を通じてほとんど均一に全層に分布し、曇天の場合といちじるしく異なる。この分布は、溶存酸素量以外の要因に

* Department of Microbial Chemistry, Faculty of pharmacy, Toky College of Science, Tokyo, Japan. 東京理科大学薬学部微生物化学教室

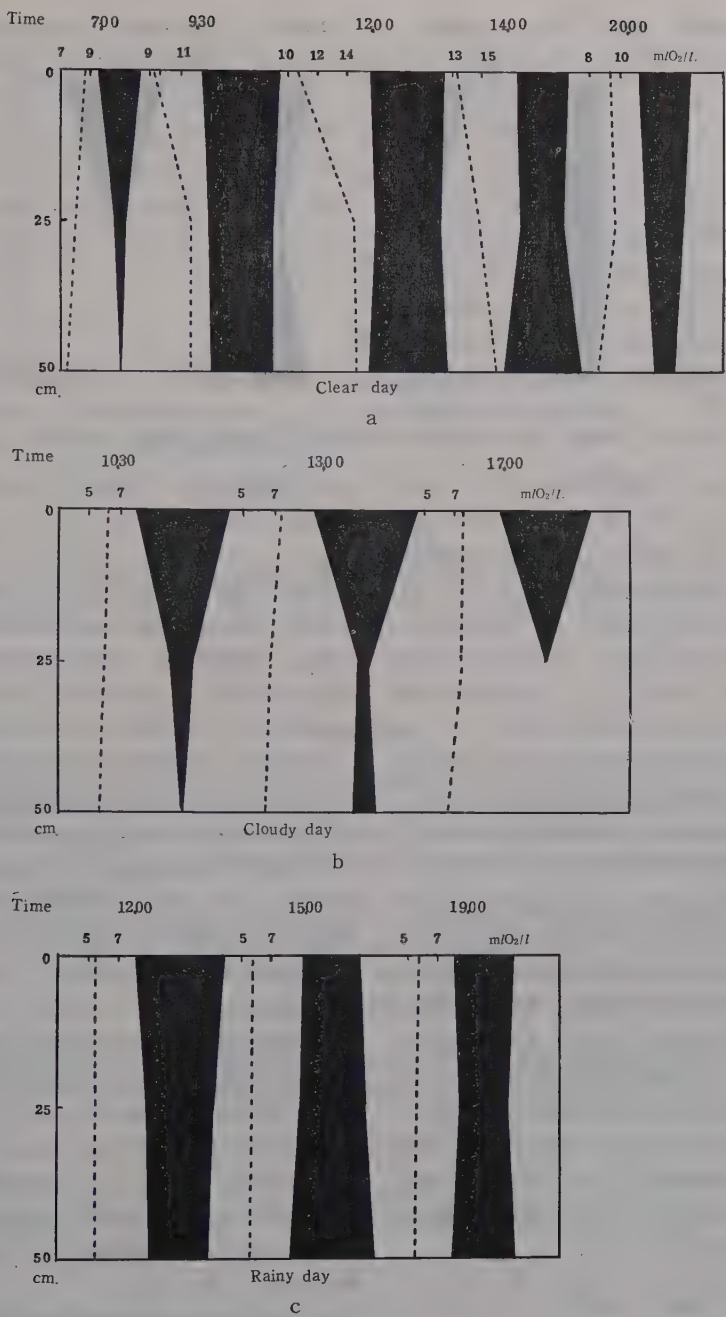


Fig. 2. Diurnal movement of zoospores of aquatic fungi in spring.

よって支配されているものと考えられる。そのなかでまず考えられるのは、水深が浅く、降雨による池水の充分な攪乱である。しかし、曇天の後の降雨のさいに、遊走子が層状分布から直ちに均一な分布に

変わる事實は、水の攪乱以外の原因が均一な分布に作用していることを示している。
水生菌類の遊走子には2本のべん毛を持って水中を遊泳する活動型と、膜をかぶって休止状態にある



Fig. 3. Diurnal movement of zoospores of aquatic fungi in winter.

休止型の2形がある。著者の観察によれば、後者は通常水面に浮いているが、降雨によって刺激され前者に変わり、水底にも分布するようになる可能性が考えられるが、今後の研究を要する。

2. 夏季および冬季

天候による差異は見られず、また遊走子の日周的な垂直移動も認められない。ここでは晴天の日の観察結果をもとにして、その概要を述べる。

両季とも溶存酸素は1日を通じて表層と中層に最も多いが、1日中遊走子の大部分は表層に集まり、まれに中層に出現するが、底層には全く見られない(第3図)。特に夏季の晴天の日には、水の華が集まる中層に最も酸素量が多いが、遊走子は中層には集まらず、酸素量の垂直分布と相関性が認められない。Salvin⁴⁾は水生菌類の第2遊泳期の遊走子は25°の水中では速い運動を行ない、30°でもなお運動力を有することを報じているが、占春池の夏季の水温は24—27°で、遊走子の遊泳に不適当とは考えられない。しかし、遊走子は表層にだけ分布し、明らかに不活発な状態にある。水温の低下した冬季の垂直移動も夏季の場合とだいたい同じで、一般に水温の不適当な季節には、まったく遊走子の垂直移動は見られないものといえよう。

考 察

上述のように水生菌類の遊走子は、動物プランクトンと同様に日周的な垂直移動を行なうことは明らかであるが、移動の様子は季節によっても、天候によっても異なる。遊走子の垂直移動を起させる要因として種々考えられるが、そのなかでも水の溶存酸

素量が支配的である。同様なことは手賀沼(千葉)⁵⁾や鶉取池(長野)⁶⁾などの浅い池沼でも観察されている。

遊走子の垂直移動を考える場合、遊走子の運動力が問題となる。Salvin⁴⁾によれば *Saprolegnia*, *Achlya*, *Dictyuchus* の第2遊泳期の遊走子の泳ぐ速度は水温によって異なるが、一般に高温ほど速く、20—25°では約200 μ /秒であるという。また、Höhnk²⁾, Cotner³⁾, Salvin⁷⁾らの研究では、遊走子の1回の遊泳時間は約1時間前後で、運動距離は50—70 cm程度となる。これを数回くり返すので、遊走子自身の運動によって分布が決定するのは水深が2—3 mの浅い池沼に限られる。実際に、水深が8.5 mの震生湖(神奈川)で水生菌類の遊走子の垂直分布の日変化を調べたが、良好な結果は得られなかった。

水生菌類の遊走子は運動力を有する活動型と、膜をかぶって休止状態にある休止型の2型が交互に現われる。Höhnk²⁾, Cotner³⁾によれば、この両型は外界条件によっても異なるが、おのおの30—100分前後でくり返えられるという。著者の観察では、休止型の遊走子は通常水面に浮く傾向が見られ、湖沼での垂直分布もこの形態変換によって影響されると考えられる。

遊走子の日周的な垂直移動が季節により異なることは、特に注目すべき現象である。遊走子の移動は、水温が遊走子の活動に良好である春季と秋季に活発で、いちじるしい垂直移動が認められるが、水温の不適当な夏季と冬季には酸素量の垂直的な差異が存在しても、遊走子の移動は見られない。これは遊

走子が不活発な休止型にあるものと考えられる。Salvin³⁾は水生菌類の第2遊泳期の遊走子は30°でも運動性を示すが、5°ではほとんど運動が見られないことを観察している。占春池の水温は夏季に25—28°, 冬季に3—5°で、冬季に遊走子が運動性を示さないのは低温によるものと考えられるが、夏季に移動の見られないのは、これによつては理解できない。

要 約

水深の浅い占春池において、水生菌類の遊走子の日周的な垂直移動について観察を行なった。

春季と秋季には、遊走子の分布は溶存酸素量の垂

直分布の日変化と相関を示し、酸素の多い層に遊走子が集まる傾向が見られた。晴天には時刻によつて分布が異なり、曇天には1日を通じて常に表層に多く、雨天には遊走子は1日中全層ほとんど均一に分布している。

夏季と冬季には、遊走子の分布は天候に無関係で、常に表層に最も多く、中層にも少数は見られるが、底層にはほとんど分布せず、溶存酸素量の垂直分布と相関性が認められない。

終りに本研究に対して、指導と助言をいただいた東京教育大学の印東弘玄、伊藤洋両教授ならびに市村俊英講師に深く感謝する。

文 献

- 1) 鈴木静夫, 日生態会誌 10: 172 (1960).
- 2) Höhnk, W., Amer. Journ. Bot. 20: 45 (1933).
- 3) Cotner, F.B., Amer. Journ. Bot. 17: 511 (1930).
- 4) Salvin, S.B., Mycologia 33: 592 (1941).
- 5) Suzuki, S., Unpublished.
- 6) ———, Unpublished.
- 7) Salvin, S.B., Mycologia 32: 148 (1940).

Summary

The diurnal migration of the zoospores of aquatic fungi was studied in Lake Senshun-ike. The distribution of the zoospores had close correlation with the diurnal vertical distribution of the dissolved oxygen in spring and autumn. In both seasons, the zoospores assembled in the more oxygen-rich layer. The feature of the diurnal migration of the zoospores differed with different weather.

In clear day, a large number of the zoospores were seen in the surface water during the night, while they were seen most abundantly in the bottom water during the day time. In cloudy day, they were found abundantly in the surface layer all the day. In rainy day, they were distributed homogeneously from the surface to the bottom layers for the whole day.

On the other hand, the diurnal migration of the zoospores of aquatic fungi was not observed in summer and winter. They were found in the surface layer through the day.

花粉の生理学的研究

主として発芽におよぼす微量元素の影響

高 見 亘*

Wataru TAKAMI: Physiological Studies of Pollen, Chiefly, the Effect of Trace Elements on the Germination of Pollen Grains.

1960 年 7 月 29 日受付

花粉の発芽に対するホウ素の影響がSchmucker¹⁾によって発見されてから、Bobko & Zerling²⁾, Cooper³⁾, Loo & Hwang⁴⁾筆者⁵⁾, 最近では山田⁶⁾の研究があるが、水耕の場合に使うように、多くの微量元素を同時に使った研究はほとんどなされていない。また、O'Kelley⁷⁾によって種々の糖液における花粉管の伸長の良否が研究されたが、糖によるホウ素の最適濃度および量が明らかでない。種子の発芽におけるホウ素の影響については Jensen⁸⁾の研究がある。花粉は発芽時にははなはだ活性の高い代謝状態にあるから助酵素の作用の上から考えても、種子の場合と同様に微量元素の影響を受けると考えられる。最近 Bogen⁹⁾その他によって非浸透的吸水が唱えられているので、この実験でみられたように水または糖液だけでは破裂する花粉も、培地に適量の微量元素を加えるとよく発芽することは、花粉の破裂は従来考えられているような単に浸透圧によるものだけではないと考えられる。ここには多くの微量元素の影響ならびに諸種の糖液において微量元素の最適濃度がちがうかどうか、さらにアスパラギンと併用した結果などを述べる。この結果は、花粉の人工的発芽の実験では寒天などを使わないだけに、添加物質の影響などを調べる場合には便利であろう。

実験の方法と結果

発芽の方法は微量元素を加えるために、主として Van Tieghem の湿室をもちいたが、場合によっては 1.5%の寒天板を使った。微量元素の影響は再蒸留水をもちいなくても、ふつうの蒸留水だけで十分見られるので、取り扱いを簡単にするために、蒸留

水 (pH 6.0) をもちいた。微量元素の原液は Bonner & Galston¹⁰⁾の著書を参考にしてつぎのように作った。

FeCl ₃	0.05 g
H ₃ BO ₃	0.05 g
MnCl ₂ ·4H ₂ O	0.03 g
ZnSO ₄	0.05 g
CuSO ₄ ·5H ₂ O	0.05 g
H ₂ MoO ₄ ·4H ₂ O	0.002 g
蒸 留 水	100 cc

この原液を培地に 1/200~1/20 の範囲で加え、一定時間後に 20 個以上の花粉管の長さを 150 倍でマイクロメーターで測り、はなはだ長くなった場合には 50 倍で描画後カーブメーターでその長さを測った。

1. 微量元素の花粉管伸長におよぼす影響: ムラサキツユクサの花粉は浸透圧かまたは微量元素の含量が変化するためか発芽の状態が変化するため¹¹⁾この実験中でもある花粉は 8% しょ糖液でも破裂が多いのに、他の株の花粉は水でも容易に発芽し、同じ株の花粉でも時期によりこのようなことが観察された。花期のはじめには水では破裂するが、後期には水でも発芽するものも多いようである。この実験に主として使用したムラサキツユクサでは 8% しょ糖液が最適であるが、1959 年 6 月 12 日には 8% しょ糖液でも破裂が多かったのに 7 月 7 日には破裂が少なかった。前者の場合に、室温 20° で 8% しょ糖液を対照として上記の原液を 1/200, 1/100, 3/200, 1/50, 1/25, 1/20 ずつ加えた培地に、同じムラサキツユクサの花の花粉をまき 15~25 分後に観察してみると対照では破裂がはなはだしいが、1/200~1/100 添加のものはよく発芽して花粉管の伸長も大きかった (表 1)。ムラサキツユクサでは悪影響がみられる濃度は花粉によって異なるが、原液を 1/50

* Biological Institute, Waseda University, Tokyo, Japan. 早稲田大学生物学教室

Table 1. Effects of different concentrations of the basal trace element solution on the pollen tube growth with 0.23 M sugar on the basis of the media (12 units correspond 130 μ).

Sugar Conc.	<i>Tradescantia reflexa</i> (22°, 15 min.)		<i>Impatiens balsamina</i> (20 min.) Red flower (25°) White flower (31°)		<i>Thea sinensis</i> (25°, 60 min.)	<i>Hypericum chinense</i> (24°, 90 min.)
	Sucrose	Glucose	Sucrose	Sucrose	Sucrose	Sucrose
0.005	6.1	7.1	12.4	11.4	16.5	7.0
0.010	8.2	7.5	16.5	17.7	18.8	7.3
0.015	5.3	1.0	15.9	16.8	20.2	9.5
0.020	4.5	burst	5.2	1.3	13.3	not observed

Table 2. Effects of Fe & Zn in 8% sucrose solution on the pollen tube growth of *Tradescantia reflexa* after 20 min. expressed in percentage of the growth in 8% sucrose solution containing 0.01 basal trace element solution.

Conc.	Fe 2 $\times 10^{-6}$ 10 $^{-6}$		Zn 1.25 $\times 10^{-6}$ 5 $\times 10^{-7}$		Fe & Zn 2 $\times 10^{-6}$ 1.25 $\times 10^{-6}$	
%	59	65	burst	almost burst	71	

添加すると悪影響が著しく、1/20 添加するとほとんど発芽しない。

なお、水で発芽したムラサキツユクサの花粉では pH 4.8 が限界で、それ以下にさがると破裂したが、pH 4.0 の水でも原液を 1/100 添加し、室温で培養すると少数の発芽がみられた。

ムラサキツユクサ以外に観察した花粉と培養時間はホウセンカ 20 分、チャ 60 分、ビョウヤナギ 90 分で、室温で発芽させ花粉管の長さを測った結果は表 1 のようである。これによって原液を 1/100~3/200 添加するときに発芽が最も促進されることがわかる。この表のホウセンカの結果は 9 月に観察したものであるが、ホウセンカのように発芽状態で変動の多いものでは、7 月 17 日 26°, 20 分後で、対照を水とした場合には原液 0.01 添加で花粉管伸長の促進効果は 30% ではなはだ大きい、8% しょ糖液を対照とすると 2% で、ほとんど影響がないといえる。

つぎに、6 月にムラサキツユクサとビョウヤナギの花粉を約 24° の室温で 8% しょ糖液を対照として数時間培養し、なるべく長い数個の花粉管の長さを測るとムラサキツユクサでは対照、原液 1/200 添加、1/100 添加の順に長さの比はおおよそ 100 : 100 : 135 となり、ビョウヤナギでは対照、原液 1/200 添

加の順に 100 : 280 となつてともに促進作用が認められた。

2. 微量元素の好影響がみられる花粉： 上の実験結果から原液を培地の 1/100 添加すると発芽が促進されることがわかったので、しょ糖液だけを培地にしたのではよく発芽しないが、微量元素添加で破裂が多少なりとも防げるものを探してみた。まだ多種のものについて検討はしていないが、好影響がみられたものに図 1-3 に示すようなものがある。これによって 8% 程度のしょ糖液で発芽可能の多くの花粉は、微量元素原液を 0.01 程度添加すると発芽が促進されることが知られる。図に示してはないうが、水で発芽するネコヤナギの花粉も、原液 1/100 添加の水にまいた方が花粉管の伸長が大きく、水では破裂するチューリップの花粉も、ある場合には、原液 1/100 添加の場合によく発芽した。エニシダの花粉も 26°, 10% しょ糖液では破裂するが、原液添加の場合には発芽がみられた。

3. 単独元素の影響と必須元素： 8% しょ糖液に微量元素原液を 1/100 添加したものを対照としてホウ素以外の微量元素 1 種ずつを対照内のそれぞれの濃度（すなわち Fe は 5 $\times 10^{-6}$, Mn は 3 $\times 10^{-6}$, Zn, Cu は 5 $\times 10^{-7}$, Mo は 2 $\times 10^{-7}$ ）になるようにした 5 種の培地にムラサキツユクサとビョウヤナ

Table 3. Effects of boron only and of all trace elements including boron on the pollen tube growth with 8% sucrose solution on the basis of the media (12 units correspond 130 μ).

Trace elements	<i>Tradescantia reflexa</i>					
	20°, 15 min.			20°, 35 min.		
	0.005 B	0.005 All	0.01 All	0.02 All	0.005 B	0.005 All
Length	4.8	5.6	7.5	3.6	15.0	15.0

Trace elements	<i>Hypericum chinense</i> 24°, 90 min.					<i>Lilium longiflorum</i> 25°, 120 min.	
	0.005 B	0.005 All	0.01 All	0.015 B	0.015 All	0.01 B	0.01 All
Length	7.1	7.0	7.3	7.0	9.5	35.2	36.5

Table 4. Effects of various B containing compounds on the pollen tube growth of *Tradescantia reflexa* after 20 min. and *Hypericum chinense* after 90 min. at 25° (12 units correspond 130 μ).

Compounds	Conc.	<i>Tradescantia reflexa</i>	<i>Hypericum chinense</i>
H ₃ BO ₃	5 \times 10 ⁻⁶	11.3	8.4
Na ₂ B ₄ O ₇	5 \times 10 ⁻⁶	10.9	7.7
NaBO ₂	5 \times 10 ⁻⁶	12.9	9.5
NaBO ₂ ·4H ₂ O	5 \times 10 ⁻⁶	8.6	8.4

ギの花粉をまくと、どの場合にも著しい悪影響がみられ、最も影響の少ないものは Fe であった。そこで Fe, Zn の濃度を小さくして試みると表 2 のようになり、単独では濃度を小さくしないと悪影響があり、適当な濃度の Fe, Zn の添加により対照よりは悪いが 8% しょ糖液よりははだよいことが知られた。多くの微量元素を同時に添加することにより拮抗作用が働くものと考えられる。

ホウ素が好影響を与えることは周知のとおりであるが、ホウ素のみと他の微量元素全部を含んだ 2 種の培地にムラサキツユクサとビヨウヤナギの花粉をまいた結果は表 3 のようで、ムラサキツユクサでは 20 分後ではホウ素だけより全部添加の方がより促進されるが、35 分後には変わらなくなり、ビヨウヤナギでは 0.005 では変わらないが、0.015 では全部添加の好影響がみられた。また、テッポウユリではあまり影響がいちじるしくはないが、古いきり花の花粉で調べるとホウ素のみでは破裂がみられるのに、全部添加した方では破裂がみられなかった (図 3, 25-27)。

ホウ素化合物 4 種 ホウ酸, ホウ砂, メタホウ酸, 過ホウ酸ナトリウムを 5 \times 10⁻⁶ 含んだ 8% しょ糖液にム

ラサキツユクサとビヨウヤナギの花粉をまいた結果は表 4 で、どちらの場合にもメタホウ酸 (NaBO₂) がホウ酸より好影響を与え、最も悪影響を与えたのはムラサキツユクサでは過ホウ酸ナトリウム (NaBO₃·4H₂O), ビヨウヤナギではホウ砂 (Na₂B₄O₇) であった。

最後に、8% しょ糖液に微量元素原液を 1/100 添加したものを対照としてホウ素以外の微量元素 1 種ずつ、またはホウ素と他の一つの 2 種ずつを除き、他の濃度は対照のと同じにした 10 種の培地にムラサキツユクサとビヨウヤナギの花粉をまいた結果は表 5 のようである。ホウ素が存在しない場合には両方の花粉において、Fe, Mn, Zn が必須元素で、ホウ素が存在する場合には、ムラサキツユクサでは Fe, Mn が、ビヨウヤナギでは Fe, Cu が必須元素であると考えられる。

4. 種々の糖類に対する微量元素の最適濃度: O'Kelley⁷⁾ は種々の糖にホウ素を添加して花粉管の伸長の割合を調べたが、ホウ素の濃度を糖によって変えていないようで、糖によって最適濃度が変わるかどうかを明らかにしていない。そこで、0.23 M しょ糖液に微量元素原液を 1/100 添加したものを対

Table 5. Effects of absence of particular elements on the pollen tube growth of *Tradescantia reflexa* after 20 min. and *Hypericum chinense* after 90 min. at 25°, expressed in percentage of the growth in 8% sucrose solution containing 0.01 basal trace element solution (B- means no B).

Tradescantia reflexa									
B-, Fe-	B-, Mn-	B-, Zn-	B-, Mo-	B-, Cu-	Fe-	Mn-	Zn-	Mo-	Cu-
—	—	9	23	46	52	63	91	91	118

Hypericum chinense									
B-, Fe-	B-, Mn-	B-, Zn-	B-, Mo-	B-, Cu-	Fe-	Mn-	Zn-	Mo-	Cu-
21	36	—	31	16	60	94	108	85	54

— means burst or no germination.

Table 6. Effects of different concentrations of the basal trace element solution in 0.23 M sugars on the pollen tube growth of *Tradescantia reflexa* after 20 min. at 20°, expressed in percentage of the growth in 0.23 M sucrose solution containing 0.01 basal trace element solution.

Sugar Conc.	Sugar					
	Sucrose	L-Arabinose	L-Xylose	L-Rhamnose	Glucose	Mannose
0.005	67	75	50	42	73	—
0.010	100	71	50	72	70	—
0.015	95	54	93	20	54	—
	D-Galactose	Fructose	Sorbose	Lactose	Maltose	Raffinose
0.005	72	28	—	105	87	77
0.010	75	14	—	114	146	58
0.015	48	—	—	92	—	54

— means burst or no germination.

照として、各種同濃度の糖に原液を 1/200, 1/100, 3/200 添加した 3 種ずつの培地にムラサキツユクサ、ビヨウヤナギ、テッポウユリ、コオニユリの花粉を 3 回ずつまいて花粉管の長さを比較した (表 6 ~ 9)。一時に実験されない場合には対照の実験を繰り返して規準にした。

ムラサキツユクサの場合には主として原液の 1/200 ~ 1/100 の濃度が最適であるが、キシロースではより濃いところに最適があり、花粉管の伸長率が 100 % 以上の糖はラクトース、マルトースだけで、この両者は原液を添加しない場合にもしょ糖よりよかった。なお、原液を添加しない場合の最良の結果を与えた糖はラフィノースであった。

ビヨウヤナギの場合には最適濃度は上の場合より変動し、花粉管の伸長率が 100 % 以上となった糖の種類も多かった。つぎに、テッポウユリ、コオニユリで類似の結果がみられたのはしょ糖、キシロース、マンノース、フルクトース、ソルボース、ラフィノースで、テッポウユリではグルコースの培地に原液 1/200 添加すると 1, 2 個発芽する程度でほとんど発芽しないが、コオニユリではしょ糖の場合よりは悪いが花粉管はよく伸長した。ところが、コオニユリではラムノースの培地に原液 1/200 添加すると花粉管の破裂が多く、それより濃い場合には破裂または不発芽となるのに、テッポウユリではどの濃度でも破裂せず花粉管はよく伸長した。

Table 7. Effects of different concentrations of the basal trace element solution in 0.23 M sugars on the pollen tube growth of *Hypericum chinense* after 90 min. at 24°, expressed in percentage of the growth in 0.23 M sucrose solution containing 0.01 basal trace element solution.

Sugar Conc.	Sucrose	L-Arabinose	L-Xylose	L-Rhamnose	Glucose	Mannose
0.005	96	—	25	93	25	—
0.010	100	—	50	52	125	—
0.015	130	—	34	52	153	—
	D-Galactose	Fructose	Sorbose	Lactose	Maltose	Raffinose
0.005	25	67	100	—	146	25
0.010	125	77	25	—	81	130
0.015	153	101	25	—	76	—

— means burst or no germination, * means delayed germination.

Table 8. Effects of different concentrations of the basal trace element solution in 0.23 M sugars on the pollen tube growth of *Lilium longiflorum* after 120 min. at 21°, expressed in percentage of the growth in 0.23M sucrose solution containing 0.01 basal trace element solution.

Sugar Conc.	Sucrose	L-Arabinose	L-Xylose	L-Rhamnose	Glucose	Mannose
0.005	57	—	38	43	—	28
0.010	100	—	52	72	—	50
0.015	83	—	—	59	—	67
	D-Galactose	Fructose	Sorbose	Lactose	Maltose	Raffinose
0.005	42	42	—	127	45	—
0.010	—	58	—	69	100	—
0.015	—	49	—	71	65	—

— means burst or no germination.

5. ペルオキシダーゼについて: 試料をスライドガラスの上にとり3%の過酸化水素水を滴下した後1%ピロガロールを1~2滴たらし数分後に検鏡し、黄色を呈するかどうかによってペルオキシダーゼの存在を調べた。ムラサキツユクサでは水で破裂したものや伸長が止まった花粉管にはペルオキシダーゼはなく、8%しよ糖液と原液0.01添加の両方の培地で発芽した花粉管を比較すると、後者のペルオキシダーゼがはるかに多いことが観察された。またエニシダの花粉はすでに述べたように10%しよ糖液では破裂するが、微量元素原液1/100添加によって発芽するものもみられ、後者の場合の花粉管や噴出物は前者の場合の噴出物よりも多量のペルオキ

シダーゼを含むと判断された。

6. 吸水力の比較: 室温18°のとき、ムラサキツユクサの花粉を水と微量元素原液0.01添加した水にまいて、2, 5, 7, 10, 15, 20分後に同じ花粉を600倍で写真にとることを数回繰り返し、マイクロメーターで乾燥花粉との大きさを比較した。水の場合にはまいてから7~8分後に破裂して、そのときの花粉の大きさは横だけが116%になり、縦はほとんど変化しなかった。微量元素原液1/100添加の場合には、まいてから5分後に横は116~133%に、縦は100~109%になって、8分後には早いものは発芽しはじめ、10分後には多くの花粉の縦は109%になった。水で発芽するネコヤナギの花粉につい

Table 9. Effects of different concentration of the basal trace element solution in 0.23 M sugars on the pollen tube growth of *Lilium Maximowiczii* after 100 min. at 27°, expressed in percentage of the growth in 0.23 M sucrose solution containing 0.01 basal trace elements solution.

Sugar Conc.	Sucrose	L-Arabinose	L-Xylose	L-Rhamnose	Glucose	Mannose
	D-Galactose	Fructose	Sorbose	Lactose	Maltose	Raffinose
0.005	94	—	34	60	37	34
0.010	100	11	66	9	83	60
0.015	89	74	23	—	29	67
0.005	28	37	—	16	27	—
0.010	34	60	—	20	41	—
0.015	60	36	—	11	63	—

— means burst or no germination.

て、室温 18°, 60 分後に写真をとって同様な比較をしてみると、この球状の花粉は水の場合には最大の膨脹は 117%であるのに、原液 1/100 添加の場合の最大の膨脹は 122%となり、わずかではあるが後者の方の膨脹率がより大きいと判断された。

7. 寒天板の場合における微量元素とアスパラギン添加の影響: ムラサキツユクサの花粉を 8% しょ糖、1.5% 寒天板を対照としてこれに微量元素原液を 0.0025, 0.005, 0.01, 0.02 添加した5種の培地にまいて、21°, 30 分後に花粉管の長さを比較すると、この順に、100, 176, 168, 253, 118% となり、4 時間後に花粉管の長いもの 4 個の平均を求めると、この順に 1.15, 1.15, 1.69, 1.46, 0.70 mm であった。そこでつぎには、原液 1/100 添加の 8% しょ糖、1.5% 寒天板を対照としてアスパラギンを 0.001, 0.01, 0.1, 1% 添加した5種の培地にムラサキツユクサの花粉をまいて、22°, 30 分後に花粉管の長さを比較すると、この順に、100, 115, 109, 105, 94% となり4時間後に花粉管の長いもの 4 個の平均を求めると、この順に 0.79, 0.96, 1.69, 0.51, 0.59 mm であった。ところが 25° で行なった他の 2 回の実験では4時間後に、この順に 1.77, 1.31, 1.54, 1.17, 0.59 mm および 1.41, 2.30, 1.09, 0.96, 0.64 mm となって、アスパラギン添加による影響は確定的とはいえなかった。

サツキの花粉を 8% しょ糖、1.5% 寒天板を対照として微量元素原液 0.01 添加およびそれにさらにアスパラギン 0.001, 0.01, 0.1, 1% 添加の6種の

培地にまいて 25°, 3 時間後に花粉管の長さを比較すると、この順に、100, 237, 244, 332, 290, 182% となって微量元素およびそれとアスパラギンによる促進作用がみられたが、24 時間後には最大の花粉管の長さはこの順に 3.33, 3.70, 2.00, 2.35, 2.15, 1.10 mm となって、これらの好影響はみられなかった。しょ糖寒天板では微量元素とアスパラギンの促進作用は発芽の初期に限られるようである。

結 論 お よ び 論 議

上の実験の結果によれば、植物の生長の場合に必要な役割を果たす微量元素は花粉の発芽においてもまた重要な作用を与えることがわかる。その最適濃度は最も多量の鉄、ホウ素に対して 5×10^{-6} 程度で、水耕の場合より約 10 倍大きい。ムラサキツユクサなどで観察したように花粉管の伸長は微量元素の適量を与えることによって増加されるが、発芽の初期にいちじるしい影響を与えるようである。ムラサキツユクサの花粉が後には 8% しょ糖液で発芽するのにははじめには破裂するのは、花粉の浸透圧が減少すると考えるよりも、花粉の熟成につれて微量元素含量が増加するために発芽しやすくなるとすべきであろう。

ムラサキツユクサの花粉で観察されたように、最初は花粉粒と培地との浸透圧の差による吸水が行なわれ、その速さは培地が水の場合と微量元素を含んだ水の場合とで差はなく、いわゆる消極的吸水⁹⁾であるが、微量元素によって酵素の活性が大きくなっ

た花粉粒では積極的能動的吸水がおこり、代謝が盛んになって花粉管が形成されるが、水にまかれた花粉粒は破裂してしまうと考えられる。前報¹⁾に述べたアルコールの適量を培地に添加することによって花粉粒の破裂が少なくなることも同様な現象と解釈される。

最近では酵素反応に関与する金属元素の種類と性質の知見が増しており、ホウ素が糖の移動に関連することも推定されているので¹²⁾、花粉の発芽に大きな偉力をもつホウ素の作用も花粉粒内で糖から花粉管が形成される場合に糖の移動が促進されることによって理解されよう（この実験に使用した程度のホウ酸の添加では pH の変化はほとんどないとしてよい）。上の実験では、微量元素添加によってペルオキシダーゼの活性が増加することを検証した。

また、上の実験でみたようにホウ素だけよりも微量元素全部添加した方が発芽に対する促進作用が大きい、各元素を単独で使用するとう有害な濃度でも同時に使用するとかえって有効になり、拮抗作用があることが認められた。ホウ素化合物のうちでは、ホウ酸よりもメタホウ酸の方がより有効であったが、ホウ素の適量が存在する場合には、他の微量元素一種を単独に添加すると好影響を与えるかどうかについて花粉による特異性があることがわかる。従来無機塩は花粉の発芽に対して害作用があり、したがって発芽実験には再蒸留水を使えとされているが¹³⁾、上の実験の結果によればその必要はなく、かえって適当な微量元素の適量を加えるとよいと考えられる。もっとも花粉によっては微量元素添加で影響のないものもあり、たとえば、水で発芽するツバキの花粉を水と微量元素原液 1/100 添加の 2 種の培地にまき、20°, 40 分後に花粉管の長さを測ってみると両方でちがいが認められなかった。これはツバキの花粉は微量元素の必要量をすでに含んでいるためと思われる。同じ日に水で発芽するネコヤナギの花粉について同様に調べてみると、微量元素による花粉

管伸長への促進作用が認められた。

つぎに、糖のうちでは一般にしょ糖が最もよいとされているが¹²⁾、ムラサキツユクサでは 0.23 M しょ糖液が最適のとき、同じ濃度のラクトースとアラビノースとはしょ糖程度で、ラムノース、マルトース、ラフィノースはしょ糖よりよかった。最良のものはラフィノースで、対照のしょ糖では全部破裂してしまったのにラフィノースでは破裂なくよく発芽した。

このように糖に特異性があるので、微量元素添加の最適濃度もしょ糖の場合と必ずしも一致せず表 6—9 に示したようである。マルトースでは添加が少し多すぎると急に害作用を与えることが注目される。これを O'Kelley⁷⁾ の結果と比べてみると、ラクトース、マルトースはムラサキツユクサ、ビヨウヤナギ、テッポウユリでは O'Kelley の場合と同様にしょ糖よりもよいが、コオニユリでは反対にはなはだ悪い。グルコース、フルクトースはビヨウヤナギ以外では O'Kelley の場合のようにしょ糖より悪いが、ビヨウヤナギではともによくて、他の花粉と異なった性質を示している。ガラクトースは O'Kelley のでも一定していないが、この実験でもビヨウヤナギでよかった以外他の花粉では悪かった。ビヨウヤナギは表 5 に示すように、ムラサキツユクサとは必須元素を異にするので、各種の糖に対する代謝機能も異なるものと思われる。

最後に、アスパラギンを加えたばあいにもムラサキツユクサとサツキについて花粉管の全長がどの程度まで増加するかを調べたが、めしべの長さには比較されるような決定的な良結果はみられなかった。沢田¹⁴⁾は適当なアミノ酸添加によって人工的に発芽しにくいトウモロコシの花粉管の伸長が促進されることを報告しているが、花粉管の全長については示されていない。Brink¹⁵⁾が酵母添加によってえとなめしべの長さに匹敵する花粉管の伸長がみられる人工培地を作ることは興味がある問題であろう。

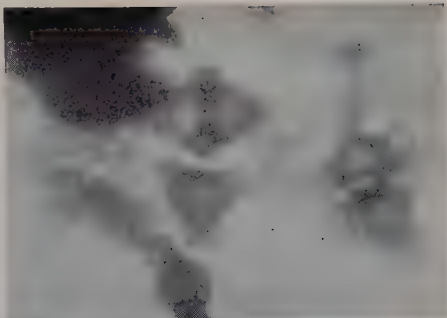
文 献

- 1) Schmucker, T., *Planta* 18: 641 (1933).
- 2) Bobko, E. V., and Zerling, V. V., *Ann. Agron.* 8: 174 (1938).
- 3) Cooper, W. C., *Bot. Gaz.* 100: 844 (1939).
- 4) Loo, Tsung-Lê Loo, and Hwang Tsung-Chen, *Amer. J. Bot.* 31: 356 (1944).
- 5) Takami, W., *Bot. Mag. Tokyo* 69: 128 (1956).
- 6) Yamada, Y., *ibid.*, 71: 319 (1958).
- 7) O'Kelley, J. C., *Amer. J. Bot.* 42: 322 (1955).
- 8) Jensen, W., *Bot. Gaz.* 113: 180 (1951).
- 9) Bogen, H. J., *Z. Bot.* 42: 153 (1954).
- 10) Bonner J., and Galston, A. W., *Principles of Plant Physiology* (1952)

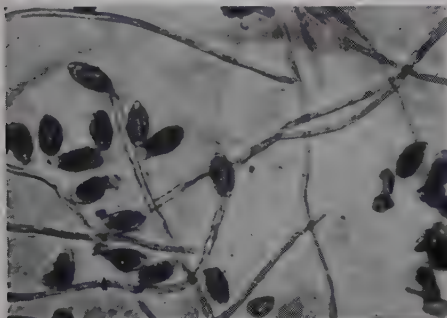
1



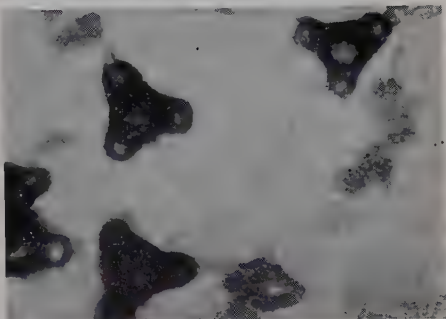
6



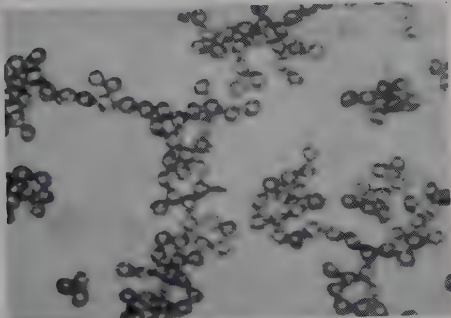
2



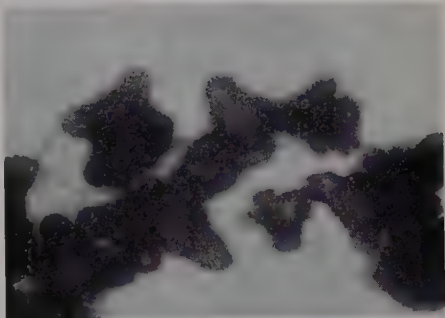
7



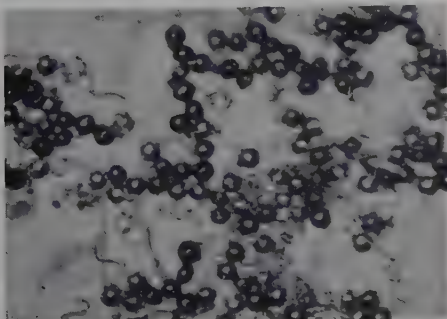
3



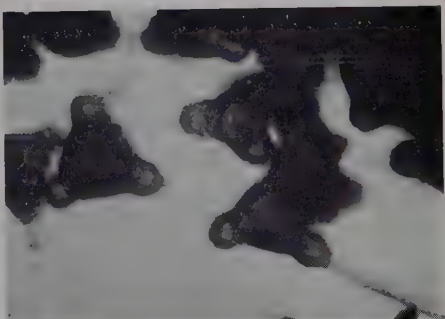
8



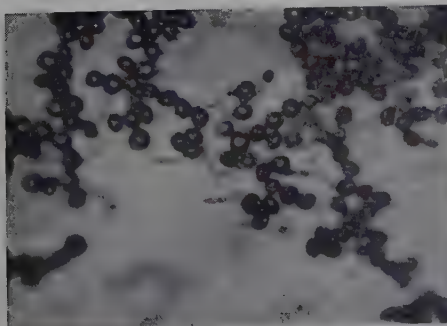
4



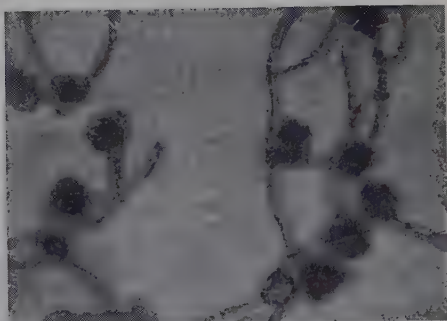
9



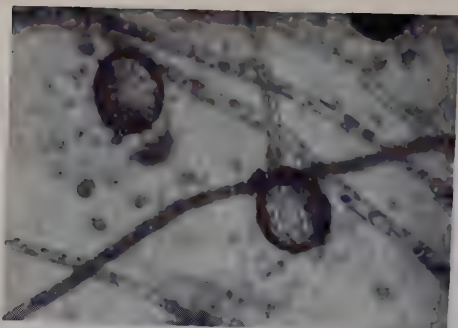
5



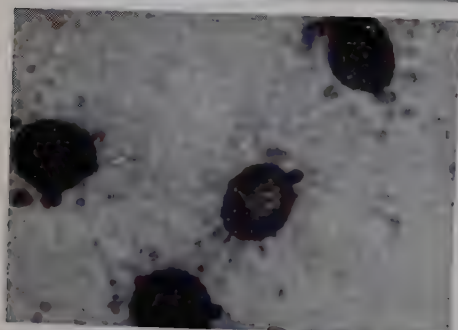
10



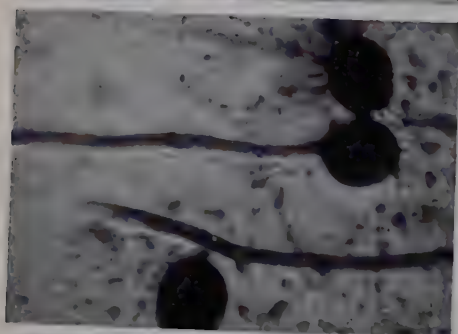
11



12



13



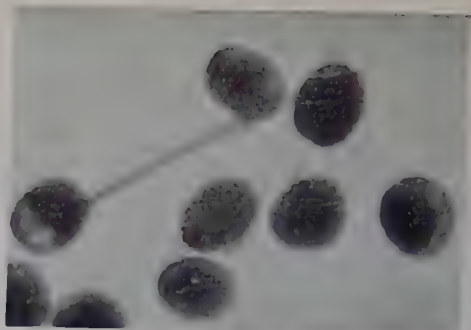
14



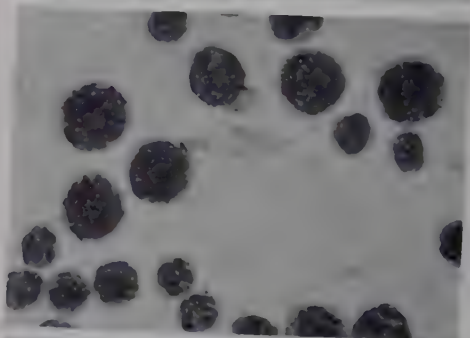
15



16



17



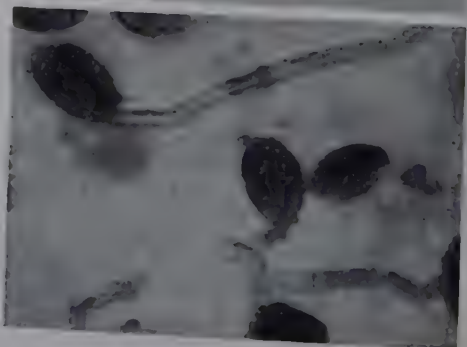
18



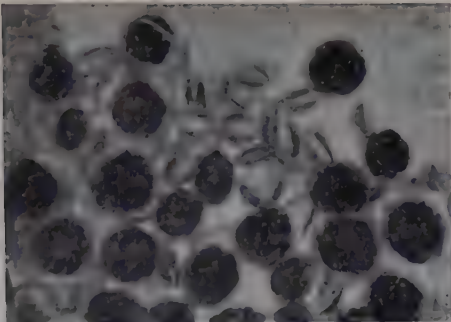
19



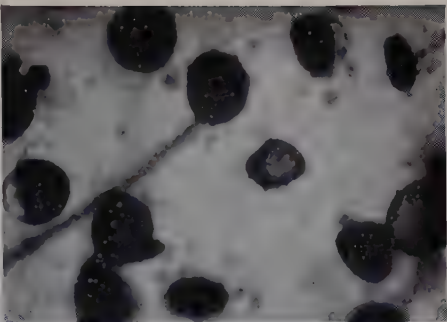
20



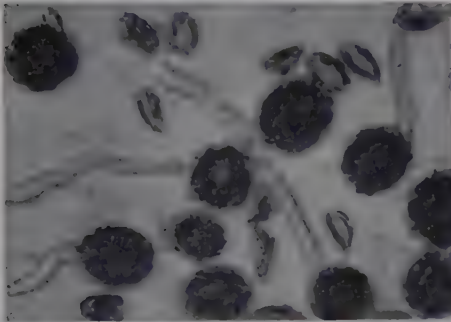
21



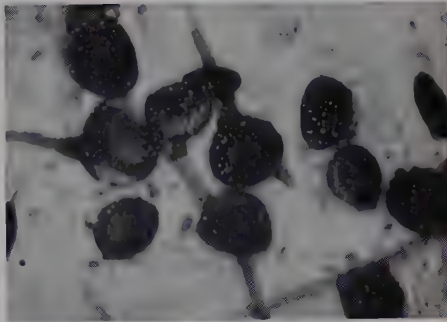
26



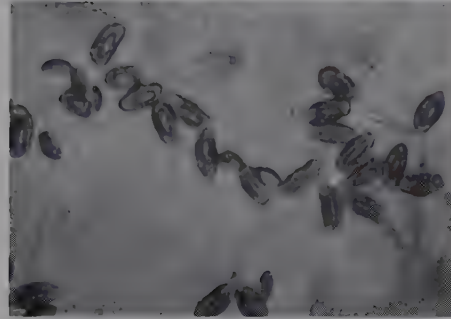
22



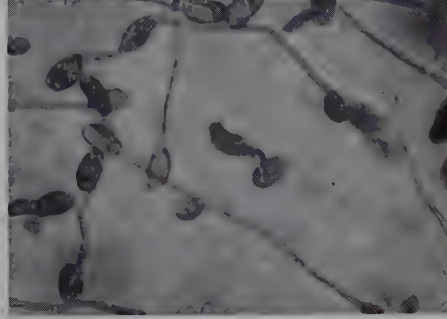
27



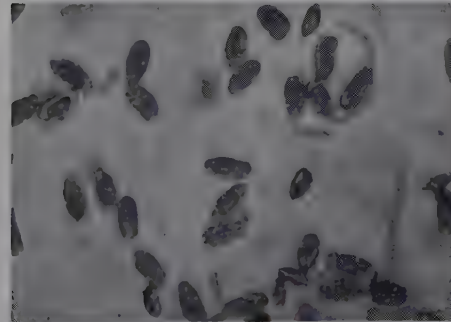
23



28



24



29



25



30

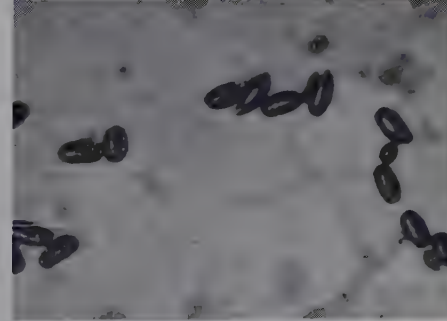


Fig. 1. Promoting effect of trace elements on pollen germination using Van Tieghem's apparatus.

1-2. *Tradescantia reflexa* after 30 min. at 24°.

1) in 8% sucrose solution, 2) in 8% sucrose solution containing 0.01 basal trace elements solution.

3-5. *Hypericum chinense* after 80 min. at 24°.

3) in water, 4) in water containing 0.01 basal solution, 5) in water containing 0.04 basal solution.

6-9. *Oenothera tetraptera* after 150 min. at 25°.

6) in 15% sucrose solution, 7) in 15% sucrose solution containing 0.01 basal solution, 8) in 20% sucrose solution, 9) in 20% sucrose solution containing 0.01 basal solution.

10. *Cucumis sativus* after 150 min. at 26°, in 25% sucrose solution containing 0.01 basal solution, while it burst in 25% sucrose solution. $\times 130$.

Fig. 2. Promoting effect of trace elements on pollen germination using Van Tieghem's apparatus (Continued).

11-14. *Lilium longiflorum* after 120 min. at 20°.

11) in 8% sucrose solution, 12) in 8% sucrose solution containing 0.01 basal solution, 13) in 8% sucrose solution, in case of cut flowers.

15-16. *Zea Mays* after 60 min. at 24°.

15) in 25% sucrose solution, 16) in 25% sucrose solution containing 0.01 basal solution.

17-18. *Calystegia japonica* after 150 min. at 26°.

17) in 25% sucrose solution, 18) in 25% sucrose solution containing 0.01 basal solution.

19-20. *Hemerocallis disticha* after 180 min. at 30°.

19) in 10% sucrose solution, 20) in 10% sucrose solution containing 0.01 basal solution. $\times 130$.

Fig. 3. Promoting effect of trace elements on pollen germination using Van Tieghem's apparatus (Continued).

21-22. *Tritonia crocosmaeflora* after 80 min. at 26°.

21) in 10% sucrose solution, 22) in 10% sucrose solution containing 0.01 basal trace element solution.

23-24. *Tradescantia reflexa* after 15 min. at 22°.

23) in 8% sucrose solution containing 5×10^{-6} H_3BO_3 , 24) in 8% sucrose solution containing 0.01 basal trace element solution.

25-27. *Lilium longiflorum* after 120 min. at 25°.

25) in 8% sucrose solution, 26) in 8% sucrose solution containing 5×10^{-6} H_3BO_3 , 27) in 8% sucrose solution containing 0.01 basal trace element solution.

28-30. *Tradescantia reflexa* after 30 min. at 22°.

28) in 0.23M lactose solution, 29-30) in 0.23M maltose solution containing 0.01 and 0.015 basal trace elements solution respectively. $\times 130$.

- 11) Takami, W., Bot. Mag. Tokyo 72: 108 (1959). 12) 生化学講座, No. 9, 植物の生化学 (1957). 13) 安田貞雄: 高等植物, 生殖生理学 (1944). 14) Sawada, Y., Bot. Mag. Tokyo 73: 252 (1960). 15) Brink, R.A., Amer. J. Bot. 11: 283 (1924).

Summary

(1) The addition of adequate amount of trace elements such as B, Fe, Mn, Zn, Cu and Mo to the culture medium showed a remarkable promoting effect on germination of pollen grains of many kinds of plants and a definite suppressing effect on bursting of pollen grains in a hypotonic medium. Promoting effect of all trace elements together was greater than that of boron alone in case of *Tradescantia reflexa*, *Hypericum chinense* and *Lilium longiflorum*. Concentration of trace elements optimal to the germination was about 10 times greater than that in the case of water culture and it lay in the range of 0.5~1.5% basal trace element solution.

(2) Effect of absence of particular trace element and comparative effect of boron compounds were examined. Sodium metaborate was more effective than boric acid in *Tradescantia reflexa* and *Hypericum chinense*.

(3) Effect of the addition of asparagine to a solution of trace elements was observed with *Tradescantia reflexa* and *Rhododendron lateritium*.

(4) Optimum concentration of trace elements in the culture media containing various kinds of sugars were determined with *Tradescantia reflexa*, *Hypericum chinense*, *Lilium longiflorum* and *Lilium Maximowiczii*.

(5) Activity of peroxidase and of water uptake became greater by the addition of adequate amount of trace elements to the culture medium.

子実層分離法による帽菌類の菌糸の培養

広 本 一 由*

Kazuyoshi HIROMOTO: Culture of Mycelia from the Fruit Body of Hymenomycetes by "The Hymenium-Isolation Method"

1960 年 8 月 23 日受付

従来帽菌類子実体から菌を分離する場合には通常菌柄内部を使用している。著者は主として松林に発生する帽菌類 19 種について、子実体各部から菌の分離を試験した結果、菌柄内部では不成功に終る場合でも、ひだを用いるとしばしば好結果が得られること、およびハリタケ属やアマタケ属などにおいても、その針や管口の子実層からは菌糸がよく発生することがわかった。子実層を用いて菌の分離を行なう方法を子実層分離法と名づけてここに報告する。なお本文においては今関・本郷¹⁾の分類法を採用した。

供 試 材 料

第 1 表に掲げた 19 種の帽菌類子実体を供試した。ハリタケ属やアマタケ属においては、なるべく晴天続きのもとで发育した新鮮な子実体を供試するように努めた。

試 験 方 法

子実体の菌柄内部、傘肉および子実層部から、あらかじめ赤熱殺菌を施したメスで、なるべく無菌的に小片を切り取り、これを扁平培養基面に載せ、温度を 25° に保ち、各片における菌糸発生の状態を比較観察した。しかし傘肉および菌柄内部は虫害や細菌などのため、または形態的にそれらの部分は実験に供し得ないものもあったので、第 1 表にはところどころ空欄を生じた。また、移植片を移したと同じ培養基に担子胞子をまいて発芽試験もあわせ行なった。移植片は菌柄内部および傘肉においては長さ約 6 mm のマッチ箱形に切り取ったが、子実層部においてはなるべく小形に切り取るように努めた。

なお、マツタケおよびシメジにおいては、子実体

の貯蔵期間と菌糸発生能力の減退に関して実験を行なった。すなわちマツタケにおいては、11 月 8 日に林地で菌がわずかにその頭部を現わしているものを標識し、10 日後、8 分開きに生長したとき採取して菌柄上部からかさを切り取り、ひだを上向きにしてペトリ皿に入れてふたを施し、室温で保存し、3 日ごとにひだの一部を切り取って移植を行ない、菌糸発生の状態を観察した。この場合、菌蕾を発見してから、これを採取するまでの期間には 1 度も降雨をみなかったもので、子実層には細菌その他の微生物の付着は比較的少なかったと考えられる。細菌その他の微生物の有無は、これをジャガイモ寒天培養基に移して確かめた。また貯蔵期間中の平均室温は 13.9°、毎日の最高および最低室温の平均はそれぞれ 17.5° および 10.4° であった。ホンシメジにおいては、4 月 29 日に全開の子実体を採取し、これを 0~5° の低温において 40 日間貯蔵し、その期間中 5 日ごとに子実体各部から移植片を切り取って培養基に移し菌糸発生の状態を観察した。

この実験に使用した培養基およびその組成はつぎの通りである。すなわち、林内の地上に発生する子実体から菌の分離には松葉煎汁寒天培養基を用いた。その組成は、アカマツの青葉を細切したもの 50 g に井戸水または水道水 200 g を加えて 1 昼夜放置したものを 10~20 分間煮沸した後放置して冷却し、その汁液を水で 3~5 倍に希釈した液 100 g および寒天 2 g である (この pH 値は 4.5~5.0, を示す)。シイタケ菌の分離にはタンニン寒天培養基を用いた。その組成は、タンニン 0.15 g, 水 100 g および寒天 2 g である。ハタケキノコ菌の分離には腐朽薬煎汁寒天培養基を用いた。その組成は、よく腐朽した薬 10 g に水 200 g を加えて 20 分間煮沸し、汁過して得た褐色液 100 g および寒天 2 g である。スギヒラタケ菌の分離には腐朽杉葉煎汁寒天培養基を用いた。その組成は、よく腐朽した杉葉

* Kumage-Minami High School, Yamaguchi Prefecture, Japan. 山口県熊毛南高等学校

10 g に水 200 g を加えて 20 分間煮沸し、汙過し 第1表に示す通りである。
て得た褐色液 100 g および寒天 2 g である。

1) シイタケ, *Lentinus edodes*

ひだ・菌柄・傘肉いずれの部分からも容易にかすがい連結のある二次菌糸が発生する。移植片を培養基に移した当初には、ひだ部は他部よりも菌糸がや

試 験 結 果

1. 子実体各部から菌糸発生に関する試験結果は

第1表 帽菌類子実体各部から菌糸の発生および担子胞子の発芽試験

種 名	菌 糸 発 生 (1~2 日後)			胞子発芽	培養基	記 事	採 取 地	採取期 (1958~1959)
	ひだ	菌柄	傘肉					
シイタケ <i>Lentinus edodes</i> (Berk.) Sing.	++++	++	++	+++	タンニン 寒天	1週間後には各部の菌糸そうは略同程度に生育	山口県平生町	5月上旬
マツオオジ <i>L. lepideus</i> Fr.	+++	+++	+++	+++	松葉煎汁 寒天		山口県由宇町	6月中旬
スギヒラタケ <i>Pleurocybella porrigens</i> (Fr.) Sing.	++	/	++	±	腐朽杉葉 煎汁寒天		広島県厳島	10月下旬
ハタケキノコ <i>Agrocybe pediades</i> (Fr.) Fayod	++	++	++	-	腐朽葉煎 汁寒天		山口県田布施町	6月中旬
マツタケ <i>Tricholoma matsutake</i> (S. Ito et Imai) Sing.	++++	+	-	++++	松葉煎汁 寒天		柳井市	6月上旬 10~11月
マツタケモドキ <i>T. robustum</i> (Fr.) Ricken (sensu Imazeki)	+++	++	++	++++	"		"	10月下旬
キシメジ <i>T. flavovirens</i> (Fr.) Lundell	++	+	/	++++	"		"	10月中旬
アイシメジ <i>T. sejunctum</i> (Fr.) Quél.	++	+	/	++++	"		"	"
ホンシメジ <i>T. aggregatum</i> (Schaeff. et Secr.) Cost. et Duf.	++++	+++	+++	++	"		柳井市 山口県周東町	4月上旬 10月中旬
シャカシメジ <i>T. conglobatum</i> (Vitt.) Sacc.	++++	+++	+++	++	"		長野県大桑村 広島県向原町	9月下旬 10月中旬
ハツタケ <i>Lactarius hatsudake</i> Tanaka	++	/	/	-	"	傘肉、菌柄は虫害著し	柳井市	10月中旬
ヌメリササタケ <i>Cortinarius mucifluus</i> (Fr.) Fr.	++	+	-	-	"		"	"
ヌメリイグチ <i>Suillus luteus</i> (Fr.) S.F Gray	+++	++	++	-	"		"	"
アミタケ <i>S. bovinus</i> (Fr.) Kuntze	+++	++	++	-	"		"	"
コウタケ <i>Sarcodon aspratus</i> (Berk.).S.Ito	+++	-	-	++++	"	細菌多し、かすがい連結なし(子実体菌糸にはある)	柳井市 広島県向原町	10月中旬
シシタケ <i>S. imbricatus</i> (Fr.) Karst.	++	-	-	-	"	細菌多し	柳井市	"
チャハリタケ <i>Calodon zonatus</i> (Fr.) Karst.	+++	/	/	-	"		"	10月下旬
クロカワ <i>Boletopsis leucomelas</i> (Fr.) Fayod	+++	+	-	-	"		"	"
シロカノシタ <i>Hydnum repandum</i> Fr. var. <i>album</i> Quél.	++++	/	/	±	"		山口県由宇町	"

- +: 菌糸が発生または胞子が発芽したことをあらわす。
- : 菌糸が発生または胞子が発芽しないことをあらわす。
- ±: 菌糸の発生または胞子の発芽が不確実なことをあらわす。

や密に発生するが、5~7 日後にはいずれの部分から生じた菌もほとんど同程度に生育がよくなる。本菌は乾燥に対する抵抗力が強く、子実体が乾燥してひだが強じんになったものでも、菌糸がよく発生する。担子胞子の発芽も良好である、

2) マツオオジ, *L. lepideus*

子実体の各部分から容易に菌糸が発生し、その生長も良好である。子実体を構成する菌糸およびそれから発生した菌糸にはかすがいい連結がみられる。したがって一次菌糸と二次菌糸との判別は外観的には可能である。

3) スギヒラタケ, *Pleurocybella porrigens*

ひだからかすがいい連結のある二次菌糸が無数に発生し、1 週間後には菌そうを形成する。本菌の子実体は形態的に菌柄および傘肉からは移植片を無菌的に切り取りがたいので、菌の分離にはひだ部を用いるのが最もよい。この培養基上においては、担子胞子の発芽はみられなかった。

4) ハタケキノコ, *Agrocybe pediades*

子実体の各部分からほとんど同程度に容易に菌糸を発生し、1 週間後には菌そうを形成する。担子胞子はこの培養基上では発芽しない。

5) マツタケ²⁾, *Tricholoma matsutake*

ひだからは、移植後 12 時間で無数の菌糸が密に発生し、1 週間後には白色の菌糸そうが肉眼で認められる。菌柄内部からも菌糸が発生するが、ひだの方が非常に良好である。若い菌蕾において将来生長の後ペールになる部分および傘肉からは菌糸が発生しない。

6) マツタケモドキ, *T. robustum*

ひだ・菌柄・傘肉いずれの部分からも菌糸がよく発生する。担子胞子の発芽率もマツタケ程度である。子実体から発生した二次菌糸にはかすがいい連結がなく、単胞子培養の菌糸と外観的には区別ができない。

7) キシメジ, *T. flavovirens* およびアイシメジ, *T. sejunctum*

両者ともひだから菌糸がよく発生するが、菌柄からは発生数が少ない。アイシメジの二次菌糸にはかすがいい連結があるが、キシメジにはない。担子胞子は両者ともよく発芽し、そのさい胞子はほとんど肥大しない。

8) ホンシメジ, *T. aggregatum*

ひだ・菌柄・傘肉いずれの部分からも菌糸がよく発生する。なかでもひだからは菌糸が密に発生する。担子胞子はよく発芽し、そのさいほとんど肥大しない。

9) シャカシメジ, *T. conglobatum*

ホンシメジと同様に子実体各部から菌の分離が容易にできるが、本菌の子実体は小形であるから菌の分離にはひだを用いるのがもっとも適当である。担子胞子はかなりよく発芽し、そのさいほとんど肥大しない。

10) ハツタケ, *Lactarius hatsudake*

ひだからは菌糸の発生をみたが、傘肉および菌柄内部は虫害が著しくて実験に供し得なかった。子実体を構成する菌糸およびこれから発生する菌糸にはかすがいい連結がみられない。担子胞子はこの培養基上では発芽しない。

11) ヌメリササタケ, *Cortinarius mucifluus*

ひだからはかすがいい連結のある二次菌糸が少数発生する。菌柄内部からは菌糸の発生がさらに少なく、傘肉からはほとんど発生しない。傘肉および菌柄内部は虫害がいちじるしくて供試し得ないことが多い。担子胞子はこの培養基上では発芽しない。

12) ヌメリイグチ, *Suillus luteus* およびアミタケ, *S. bovinus*

両菌とも管口・傘肉・菌柄いずれの部分からも菌糸が容易に発生する。二次菌糸にはかすがいい連結がみられない。両菌ともこの培養基上では担子胞子が発芽しない。

13) コウタケ, *Sarcodon aspratus*

傘肉および菌柄内部は虫害がいちじるしく、かりに虫害がなくても細菌が多くて菌の分離ができない。針にも多くの場合細菌が付着していて菌の分離が困難であるが、細菌が付着していない針からは菌糸が多数発生する。子実体を構成している菌糸にはかすがいい連結が明瞭に認められるが、針から発生した菌糸にはかすがいい連結がないので更にくわしく検討する必要がある。菌糸の生長は、この培養基では非常に緩慢で、その速さはマツタケ菌糸の 1/10 にも達しない。担子胞子はよく発芽し、そのさいいちじるしく肥大する。発芽率は 80 % 以上に達するが、やはりその後の生育はよくない。

14) シシタケ, *S. imbricatus*

傘肉および菌柄内部は虫害がいちじるしく、かり

に虫害がなくても細菌が多くて菌糸が発生しない。針にも細菌が非常に多いが、細菌が付着していない場合には多数の菌糸が発生して白色の菌そうを形成する。子実体を構成する菌糸およびこれから発生した菌糸にはかすがいい連結がみられない。胞子はこの培養基上では発芽しない。本菌には地中菌糸に多数の分生子がみられるが、この分生子も、この培養基上では発芽しない。

15) チャハリタケ, *Calodon zonatus*

針からは多数の菌糸が発生するが、それと同時に細菌の発生が盛んになるので菌の分離が困難である。傘肉や菌柄からは適当な移植片を切り取ることができないので、これらの部分では試験できなかった。担子胞子はこの培養基上では発芽しない。

16) クロカワ, *Boletopsis leucomelas*

管口を移植すると、まずその周辺からかすがいい連結のある二次菌糸が無数に発生するが、同時に細菌の発生が盛んになるので菌の分離が困難である。菌柄内部からは、ときにわずかに菌糸が発生するが、傘肉からは発生しない。菌柄内部および傘肉にも細菌が非常に多い。担子胞子はこの培養基上では発芽しない。

17) シロカノシタ, *Hydnum repandum*

針からは無数の菌糸が発生するが、同時に細菌が盛んに発生するので菌の分離が困難である。本菌の子実体は小形で、また傘肉や菌柄内部は虫害がいちじるしく、これらの部分については試験できなかった。

2. 子実体の貯蔵と菌糸発生能力の減退に関する実験結果は第2表および第3表に示す通りである。

1) マツタケのひだにおいては、貯蔵期間 12 日までは、菌糸の発生状態にほとんど優劣の差が認められなかった。15 日および 18 日間貯蔵したものは、菌糸の発生数がしだいに減退したが、それでも 1 週間後にはほとんどすべての移植片の周囲に白色の菌糸そうを肉眼的に認めることができた。この頃には、ひだはたがいにくつつきあってかたまっていたので、メスの先端でひだの 1 枚 1 枚をはぎとって移植を行なった。またかさの表面からは雑菌が発生して、しだいにひだ部にのびるのを認めた。21 日間貯蔵したものでは、菌糸が全く発生しない移植片もあったが、わずかに菌糸が発生したものの周囲には 2 週間後に白色の菌糸そうが肉眼的に認められ

第2表 マツタケ子実体の貯蔵期間とひだにおける菌糸発生能力の減退

培養期間 貯蔵期間	12	48	120	168 時間以上
3 日	+++	++++ +	++++ ++++	1 週間後にはすべての移植片から菌糸そうを生ず
6	+++	++++ +	++++ ++++	
9	+++	++++	++++ ++++	
12	++	++++	++++ ++++	
15	+	++	++++	1 週間後にはほとんどすべての移植片から菌糸そうを生ず
18	-	+	++	
21	-	-	+	2 週間後に菌糸そうを生ずるものもある
24	-	-	±	

符号は第1表と同じ。

第3表 ホンシメジ子実体の貯蔵期間とひだにおける菌糸発生能力の減退

培養期間 貯蔵期間	24	48	168 時間
5 日	+++	++++	肉眼的菌糸そうを形成
10	+++	++++	
15	+++	++++	
20	+++	++++	
25	++	+++	
30	+	++	"
35	+	++	
40	-	-	

符号は第1表と同じ。

た。24 日間貯蔵したものではひだの白色が淡褐色に変じ、大部分の移植片は菌糸を発生しなかったが、まれに移植後 1 週間して数本の菌糸を発生し、4 週間後には生長して白色の菌糸そうを形成するものがあった。この場合、菌糸を全く発生しない移植片をじゃがいも寒天培養基に移してみたが、細菌その他の微生物の発生はみられなかった。したがって、この実験において使用したひだは無菌的に貯蔵されて、少なくとも 21 日間は生活力を保持していたものと思われる。

2) ホンシメジ子実体を貯蔵した場合、ひだにお

いては、貯蔵期間 30 日以後は菌糸の発生数が減少し、肉眼的菌糸そうを形成するまでの日数が延びるが、菌の分離には 40 日間貯蔵したものでも好結果が得られた。この実験において菌柄および傘肉からは、40 日間貯蔵したものでも移植当初には少数の菌糸の発生をみたが、しだいに細菌の発生がいちじるしく、菌糸はついに枯死して菌の分離はできなかつた。

考 察

帽菌類 19 種の子実体において、子実層、傘肉および菌柄内部を用いて菌の分離を試験した結果をみると、子実層部を用いた場合に最も良好な結果が得られている。従来子実体から菌の分離を行なう場合には、無菌状態の部分を使用する目的のもとに、一般に若くて新鮮な子実体の菌柄内部を用いているが、若い子実体でもしばしば虫害をうけているか、かりに虫害をまぬがれていても菌柄が小形なため無菌的に移植片を切り取りにくいこともある。またこの部分から健全な組織を移植してもほとんど菌糸を発生しない場合も多いが、子実層部を用いると、子実体がかなり古くても、または相当に長期間保存しておいたものでも、多くの場合菌糸がよく発生するので、菌の分離に成功することが多い。これは子実体を構成する菌糸の最先端である子実層部において、菌糸の生活力が最も旺盛であるためと思われる。萩本・小西⁸⁾はツクリタケにおいてはひだで生長ホルモンがつくられると報告している。シイタケ、マツオオジ、スギヒラタケおよびハタケキノコ以外の種の帽菌類はいずれも林中の地上に発生するものであるが、これらはすべて松葉煎汁寒天培養基上において子実層から多数の菌糸を発生する。したがって、これら帽菌類菌糸の生育には松葉煎汁中の未知成分が重要な役割を果たしているものと考えられる。

一般に針および管口には、ひだよりも細菌が多くて菌の分離が困難であるが、これは、針や管口は子実体が幼少なときから既に外気にさらされていること、さらにまたこれらの帽菌類はクロカワ、シンタケ、コウタケなどのように、多くは腐朽落葉の下層において生長を続けるので細菌が付着し易いことな

どによると思われる。このような場合でも子実層部以上に菌の分離に適した部位はない。シイタケ、スギヒラタケ、ハタケキノコなどの腐生菌やマツタケモドキ、アマタケ、ヌメリイグチ、ホンシメジおよびジャコシメジなどでは、子実層部のほか、傘肉や菌柄内部からも菌糸がよく発生し、その生長も比較的速い。したがってこれらの菌の生育にあたっては、マツタケほどに菌根形成を必要としないのではないかと思われる。シメジについて増井⁴⁾はマツと、伊藤⁵⁾はコナラと菌根を形成すると発表しているが、著者はシメジがアカマツ林および広葉樹林のいずれにも発生する事実を認めている。またシメジ菌は種々の培養基上によく生育し、腐植質培養も比較的容易にできる。これらの事実は本菌が腐生菌に近いことを裏づけるもので、これらの菌の人工増殖は比較的容易にできるのではないかと思われる。

摘 要

1) 19 種の帽菌類子実体の各部から菌の分離実験を行なった結果、菌の分離に最適の部位は子実層部であることが明らかになった。この部位を用いて菌の分離を行なう方法を子実層分離法と名づける。

2) 林地に発生する帽菌類に子実層分離法を適用するさいに適当な培養基は、多くの場合松葉煎汁寒天培養基である。

3) 菌柄内部や傘肉は、虫害が多いかまたは子実体が小形であるために、移植片を無菌的に切取りにくいことがある。この場合に子実層分離法は最も重要であり、そして一般に好結果が得られる。

4) 傘肉および菌柄からは菌糸が発生しないか、たとえ発生してもそれが少数のため菌の分離が困難な場合が少なくないが、子実層からは多くの場合、多数の菌糸が密に発生する。

5) 子実層分離法はひだの場合に最もよい結果が得られる。針や管口の場合には、細菌の発生により菌の分離に困難をとまることが多いが、この場合でも菌柄や傘肉に比して菌糸の発生が良好である。

謝 辞

種々御助言、御教示をいただいた京大助教授浜田稔博士に深く感謝する。

文 献

- 1) 今関六也・本郷次雄, 原色日本菌類図譜, 東京 (1957). 2) 広本一由, 植雑 73: 326 (1960). 3) 萩本 宏・小西通夫, 植雑 72: 359 (1959). 4) Masui, K., Memo. Coll. Sci. Kyoto. Imp. Univ. Series B. Vol. III. No. 2: 149 (1927). 5) 伊藤一雄, 林学会報 23: 124 (1941).

Summary

(1) Experiments on nineteen species of hymenomycetous fungi showed that the best part in isolating the mycelium from the fruit body is that of hymenium.

(2) When this "Hymenium-Isolation Method" was applied to the hymenomycetous fungi which grow on the forest land, a suitable culture medium was pine-needle decoction agar.

(3) It is often impossible to use the part of pileus flesh or stipe tissue for isolating the mycelium, for being small or often damaged by insects. In such cases, a desirable result can be obtained by applying "The Hymenium-Isolation Method".

(4) There are not a few cases where hyphae do not grow out from the part of pileus flesh or stipe; and even if the hyphae grow out, they are scanty in number and their isolation is sometimes difficult. However, when the part of hymenium is used, a luxuriant growth of hyphae can often be seen.

(5) In adopting "The Hymenium-Isolation Method", the best result can be obtained when the gill is used. When the needle or the tube is used, it is sometimes difficult to isolate the mycelium because of bacterial contamination. Nevertheless the needle or the tube is superior to the part of pileus or stipe in a same fruit body.

本 会 記 事

会長選挙

会則第9条および付則第3第1条によっておこなわれた会長選挙の投票は2月末日に締切り、3月1日に高宮篤氏の立会で開票しました。その結果服部静夫氏が当選され、昭和38年3月31日まで会長の任にあたられます。

開票結果は次のとおりです。

服 部 静 夫	280
芦 田 譲 治	142
和 田 文 吾	109
そ の 他	25
<hr/>	
総 投 票 数	556

昭和35年度会計決算報告 (昭和35年1月1日—昭和35年12月31日)

収 入 の 部		支 出 の 部	
会 費	1139,569	出 版 費	1273,654
予 約 購 読 料	514,387	発 送 費	118,044
バックナンバー	49,435	編 集 関 係 費	101,633
別 刷 代	181,608	図 書 関 係 費	9,763
文部省刊行補助	180,000	庶 務 関 係 費	203,027
利 子	12,084	大 会 関 係 費	50,000
広 告 料	29,000	支 部 補 助	26,000
		幹 事 手 当	160,500
小 計	2106,083	小 計	1942,621
前年度繰越金	388,256	次年度繰越金	551,718
総 計	2494,339	総 計	2494,339

Developmental Mechanics of Fucaceous Algae XVIII. Localization of Protoplasmic Elements in the Developing Rhizoid

by Singo NAKAZAWA*

Received June 9, 1960

It was described by some authors that brown plastids were localized in the distal zone of the rhizoid of fucoid embryos¹⁻³). In addition, the present writer has noticed that there are some other globules of a pale color localized in the proximal region of the young rhizoids, though these have not always been described clearly by the former authors. Under normal conditions, they are localized at their peculiar regions without any dislocation. This stability in localization seems to be related to the development of the rhizoid. Therefore, experiments were undertaken to disorder the localization of these elements contained in the young rhizoid.

Material and Method

Young embryos of *Coccophora Langsdorfii*, which were just differentiating rhizoids, were cultured in glass vessels with normal sea water and used as experimental material. The experiments were carried out at the Marine Biological Station of Asamushi in April, 1958 and 1960. The material was put into the centrifuging tube with sea water and was centrifuged at 25,000 times gravity for five minutes. After this, the embryos were taken out of the tube, observed, and cultured for inspection of further development. Prussian blue reaction and reduction of Fehling's solution were tested for the pale globules contained in the rhizoid.

Results with Discussion

Usually, the cells composing the main part of the embryo contain brown plastids, nucleus, pale globules and some other elements. Among these, the most conspicuous are the plastids. The site of the nucleus is discernible by the presence of a semi-transparent area where the brown plastids are excluded. The pale globules are hardly observable as they are hidden amid the plastids. In rhizoids, the plastids are less in number and are localized at the distal zone (Fig. 1A). The nucleus is obscure in the rhizoid, but it can be detected usually in the middle part. The pale globules are localized at the basal region.

When centrifuged, the pale globules are stratified at the centripetal end of each cell composing the embryo. But as they are rather few, their presence is not always clear, so that the next layer, i. e. the plastid layer, usually comes to the centripetal end. As a result, the boundary of each cell becomes conspicuous as it is bordered by the stratified layer of plastids (Fig. 1B-D). The later the developmental stage is at which it is centrifuged, the more obscure the presence of the pale globules becomes. Therefore, if the embryo is centrifuged earlier, say at three- or four-cell

* Biology Department, Yamagata University, Yamagata, Japan.

stage, or just after the first cleavage, the pale globules are stratified abundantly at the centripetal end. Thus, they may be supposed to be the same globules which are separated as the so-called 'oil cap' by centrifuging before the start of development^{4, 8, 9)}. Recently, Ando^{5, 6)} described similar globules under the term 'physodes' with the remark that they were of lipid nature. Hence, it seems that the pale globules originate from those globules which move to the centripetal end when the egg is centrifuged.

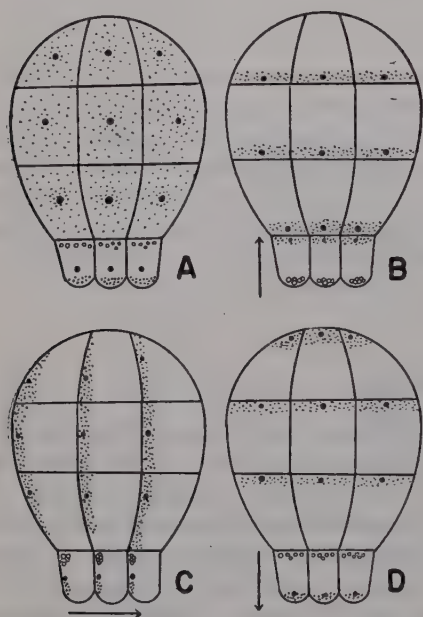


Fig. 1. A, normal embryo; B, the same centrifuged apically (proximally for rhizoids); C, the same laterally; D, the same basally (distally for rhizoids). Dots represent plastids, small circles the pale globules, and black spots the nuclei. Arrow indicates the centrifugal direction.

In rhizoids, the stratification appears a little different. When centrifuged laterally, the plastids, the nucleus and the pale globules are stratified at the centripetal end into the zone where they were located originally (Fig. 1 C). However, when the centrifugal force was exerted towards the tip of the rhizoid, the pale globules were stratified at the base of the rhizoid cell, and the plastids and the nucleus at the tip (Fig. 1 D). Consequently the original localization of the plastids and the pale globules were not altered. In other words, the pale globules moved to the centripetal end as in the main cells of the embryo, but the plastids and the nucleus were gathered to the centrifugal end not coinciding with the stratification in the embryo cells, and both layers were separated by a clear zone intervening between the two. But, when the rhizoid was centrifuged in the opposite direction, i. e. the centrifugal force was directed towards the base of the rhizoid, the pale globules were gathered to the tip, and the plastids with the nucleus to the proximal end of the rhizoid separated from the former by the intervening clear zone (Fig. 1 B). In rhizoids, as well as in the embryo cell, the nucleus always takes its position in the layer of plastids.

It is known that the physodes of *Dictyopteris* have the remarkable property of reducing Fehling's solution⁶⁾ and they are presumed to be the same element as found in fucoid cells⁷⁾. It follows that the pale globules localized to the proximal region of the rhizoid of the present material are also presumed to be the same. Here the following tests were carried out. Normal embryos were fixed with five per cent formalin for 24 hours, rinsed with water, boiled with Fehling's reagent, cooled, and inspected with a microscope. Yellow to orange particles were observed in the basal region of the young rhizoid just in or around the bodies presumed to be the pale globules. Another test, reduction of ferric ions, was performed. One tenth per cent solutions of ferric chloride, potassium ferrocyanide, and potassium ferricyanide were prepared separately. The ferric chloride solution was put in a Petri dish and was placed in the dark so that reduction by light might be avoided. Two drops of the ferric chloride solution were put on a slide glass separately; to one was added a drop of the potassium ferrocyanide solution, and to the other that of the potassium

ferricyanide. In the former, Prussian blue reaction did occur, while it did not in the latter. Thus it was ascertained that the ferric ions were not reduced to ferrous. Fresh embryos were put in the ferric chloride solution contained in a Petri dish for 20 minutes, rinsed for one minute, immersed in the solution of potassium ferrocyanide and potassium ferricyanide separately for 10 minutes, rinsed, and were inspected with a microscope. The test was carried out under a red lamp safe for gaslight paper. As a result, the embryos were blued entirely in the potassium ferrocyanide indicating that the ferric ions were taken into the cells of embryo. An interesting phenomenon occurred in the potassium ferricyanide. That is, some blue particles were observed selectively at the proximal region of the young rhizoid, showing that the ferric ions were reduced to ferrous in that part. This also implies that the pale globules, located in the same region, are taking part in the reduction. These properties of reducing metallic ions show that the pale globules are presumably a kind of physodes.

As is seen in the results of the experiments, it is evident that the specific gravity of the pale globules is smaller than those of the plastids and of the nucleus. In addition, it is also true that each of these is lighter than the hyaline matrix of the cytoplasm as is seen in the stratification when the rhizoid was centrifuged laterally (Fig. 1 C). Therefore, the pale globules are lighter than plastids and the nucleus and both the plastids and the nucleus are lighter than the hyaline matrix. The relation is as follows:

$$[\text{pale globules}] < [\text{plastids and nucleus}] < [\text{hyaline cytoplasm}].$$

If so, it is questioned why plastids are not stratified next to the pale globules but move to the centrifugal end when the rhizoid is centrifuged in the proximal or the distal direction. This is a further question. The stratification, both in the embryo and in the rhizoid, is redistributed in time to their original positions and the development of the embryo and the rhizoid proceeds normally. This implies that the localization of these different elements is controlled by the cortical layer of the cytoplasm which is hardly movable by means of centrifuging. This interpretation is advanced by the theory which insists that the morphogenetic polarity is controlled by the cortex of the protoplasm.

Summary

Young embryos of *Coccophora Langsdorfii* just differentiating the primary rhizoids were ultracentrifuged at 25,000 times gravity for five minutes. As a result, the following was discovered.

(1) The cell of normal young rhizoid contains plastids, the nucleus, pale globules and the cytoplasm matrix. The plastids are localized in the distal region, the nucleus in the middle, and the pale globules in the proximal region. When the rhizoid is centrifuged laterally, each of these elements is stratified centripetally in each zone. This indicates that their specific gravities are smaller than that of the hyaline matrix of cytoplasm.

(2) If the rhizoid is centrifuged in the proximal or in the distal direction, the pale globules are also stratified to the centripetal end, while the plastids and the nucleus are gathered to the centrifugal being separated from the pale globules by a transparent zone intervening.

(3) The pale globules are considered to be a kind of physodes, having properties reducing ferric ions and Fehling's solution.

The writer expresses his gratitudes to Prof. A. Kimura at the Tohoku University, Dr. Y. Ando at the Sapporo Institute of Hygiene, and the members of the Marine Biological Station of Asamushi for their kind cooperation in the present research.

References

- 1) Okabe, S., Sci. Rep. Tohoku Univ. 4th Ser. **4**: 591 (1929). 2) Inoh, S., J. Fac. Sci. Hokkaido Univ. Ser. 5, **5**: 9 (1935). 3) Sawada, T., Sci. Bull. Fac. Agr. Kyushu. Univ. **15**: 541 (1956). 4) Nakazawa, S., Sci. Rep. Tohoku Univ. 4th Ser. **23**: 119 (1957). 5) Ando, Y., Bot. Mag. Tokyo **64**: 192 (1951). 6) —, Bull. Jap. Soc. Phycol. **6**: 45 (1958). 7) Kylin, H., Ber. deutsch. bot. Gesell. **36**: 10 (1918). 8) Whitaker, D. M., Biol. Bull. **61**: 294 (1937). 9) Levring, T., Physiol. Plantarum **5**: 528 (1952).

摘 要

中沢信午： フークス科藻類の発生力学 XVIII. 形成しつつある仮根における
原形質要素の配分

スギモク (*Coccophora Langsdorfi*) の仮根を形成しつつある幼胚を重力の 25,000 倍で 5 分間超遠心した結果つぎのことが知られた。

(1) 正常の幼仮根には核、細胞質マトリクス、プラスチドおよび青白色小体の少なくとも 4 種の要素があり、プラスチドは主として仮根の先端部域、核は中央、青白色小体は基部に配位し、細胞質マトリクスは全面に分布している。仮根の長軸に対して横向きに遠心力を作用させるとこれらの要素のうちマトリクス以外はすべてそれぞれの部域において求心側に移動する。これはそれらの比重がマトリクスよりも小さいことを示している。

(2) 仮根に対して基部または先端向きに遠心力を作用させると、青白色小体はやはり求心端にあつまる。しかし核とプラスチドは遠心端にあつまり、青白色小体とは中間に位置する透明な部域によってはなれる。

(3) 青白色小体はおそらく *physode* の一種で、第 2 鉄イオンおよびフェーリング氏液を還元する性質をもっている。(山形大学文理学部生物学教室)

Electron-microscopical Studies on Chromoplast

I. The Ultrastructure of Chromoplast in Orange

by Masako OSUMI*

Received June 22, 1960

The structure of chloroplasts has been studied on the various plants by many investigators. Recently, by means of electron-microscope, the ultrastructure of the chloroplasts has been made clear. However, as to the structure of chromoplasts very little studies have been made.

Frey-Wyssling and Kreutzer (1958)¹⁾ classified the chromoplasts of the carotenoid-pigmented plants into three different types, namely, (1) the plastid which contains microscopic crystals of carotenoids, (2) the one which contains microscopic and sub-microscopic yellow globuli and (3) the one which contains bundles of orange-red pigmented submicroscopic filaments.

In the first group, there are carrot plastids in which pigments seem to be unbound and crystallized. In the second group of chromoplast, for instance, in *Ranunculus repens*¹⁾ or *Aloe plicatilis*²⁾, the pigments are dissolved most probably in the lipid component of the plastid. In the third group or the spindle-shaped filamentous type of chromoplasts as found in some solanaceous fruit, namely rose hips (Steffen and Walter, 1955 and 1958)^{2,3)} and red pepper (Frey-Wyssling, 1958)⁴⁾, the carotenoids appear to be bound chemically to filamentous protein structure.

According to Granick (1955)⁵⁾, the chloroplasts have generally lamellar structure. In the case of chromoplasts, however, it seems that they have not always the same, but different types of structure depending upon species.

Therefore, the present author wishes to study and compare the structures of chromoplasts in various plants. She has already studied the shape and structure of chromoplast of *Citrus* by means of ordinary microscope (Yuasa, Osumi and Nakamura, 1957)⁶⁾. In order to study the ultrastructure of the chromoplast, the electron-microscope was used in the present study.

Materials and Methods

The mature chromoplasts in the following plants were used as the materials:

Citrus unshiu (Unshu-mikan),

C. unshiu var. *praecox* (Waseunshu-mikan).

Before the observation by the electron-microscope, juice was pressed out from a fresh orange-fruit and centrifuged. The chromoplasts were gathered and fixed in 1% OsO₄ solution in acetate-veronal buffer (pH. 7.4) for 1-20 hours. After the fixation they were dehydrated in an alcohol series and embedded in *monomer*.

The sections were cut with a JUM-5 type ultra-section microtome of Japan Electron Optic Laboratory Ltd., using glass knives, and studied, in most cases, without the removal of methacrylate. An HS-6 type electron-microscope of Hitachi Ltd. was used and electron-microphotographs were taken at an initial magnification of 3000-10000 and enlarged photographically.

The observation was also made by ordinary microscope. In this case, the

* Department of Biology, Faculty of Home Economics, Japan Women's University, Tokyo, Japan.

chromoplasts in juice-sac were smeared on the slide glass and observed prior or after staining with 0.5–1 per cent aqueous solution of nigrosin. They were sometimes treated with 1–5% aqueous solution of KOH, at first, and then observed. To observe the lipid, the chromoplasts were stained with Sudan III, Sudan IV and Sudan black B.

Results

The following results were obtained on the ultrastructure of the chromoplast in the juice-sac cell of orange-fruit by examining three hundred electron microphotographs. The most chromoplasts are spindle-shaped, but occasionally the triangular or ring-shaped chromoplasts are found.

The size of the mature chromoplast is normally 15–16 μ in length and 1.7–2.3 μ in diameter.

The chromoplast seems to be covered with the membrane which is 320–340 Å in thickness. In the Fig. 1 of Plate I, the membrane seems to have split open in the course of fixing or embedding.

The chromoplast seems to be composed of many fine fibrils which are connected with each other in some portions and they look like nets (Plate I). The cross section of the chromoplast (Plate II, Fig. 2) confirms this presumption, that is to say, many small spots are seen scattering inside the chromoplast-membrane. This may mean that the fine lines in the longitudinal section of the chromoplast are not lamellae but fibrils. Each fibril is 290–380 Å in diameter and the some portions of them are thicker (1100–1600 Å) than the other portions.

There are seen several globuli among the fibrils. They are 2200–3900 Å in diameter (Plate I, Fig. 1; Plate II, Fig. 3). The globuli are supposed to be lipid from staining with Sudan black B, etc.

Discussion

By measuring with ordinary microscope, the size of mature spindle-shaped chromoplasts in orange-fruit has been confirmed to be 22–28 μ in length and 1.1–3.1 μ in diameter (Yuasa, Osumi and Nakamura, 1957)⁶.

The chromoplast of orange-fruit seems to be covered with thin membrane which is not of double-structure. In the electron-microscopical study of the chromoplast in the red pepper (Frey-Wyssling and Kreutzer, 1958)⁴, the membrane has also been shown clearly.

By means of the ordinary microscope, Yuasa, Osumi and Nakamura (1957)⁶ observed the "grana" which contain yellow carotenoid in the chromoplast of mandarin orange. The thicker portions of the fibrils which are shown by the electron-microscope are thought to be the "grana". In the chromoplast of orange-fruit carotenoid is thought to be contained in the thicker portions of the chromoplasts.

Observed with the ordinary microscope lipid granules are found in the chromoplast, which are stained with Sudan III, Sudan IV or Sudan black B. The globuli among the fibrils in the electron-microphotograph will agree with the ones which are observed by ordinary microscope. According to the report by Frey-Wyssling and Kreutzer (1958)¹, the contents which are characteristic to the yellow plastid of *Ranunculus repens* are homogeneous osmophilic globuli up to 1500 Å in diameter which first appear in young chromoplast or leucoplast; they are formed between the lamellae and, while increasing in size and number, they destroy the lamellar structure until at maturity,

only these droplets remain lining the inner surface of the plastid membrane; it is most likely that they are formed as the result of lipophanerosis of lamellar structures. However, the globuli in the chromoplast of the orange-fruit are thought to have no direct relation with the destruction of the fibrils.

From structural point of view, the chromoplast in mandarin orange-fruit will be classified into the third type by Frey-Wyssling and Kreutzer (1958)⁴). The shape of the chromoplast is spindle and the carotenoids appear to be bound chemically to fibrous structure.

However, the shape and the length of the fibrils seem to be different depending upon the species of plant. The fibrils in the chromoplast of red pepper appear in 3 or 4 bundles and they are relatively short and are not connected with each other (Frey-Wyssling and Kreutzer, 1958)⁴). On the other hand, the fibrils in the chromoplast of present material do not appear as bundles, but they are fine and long fibrils which are connected with each other like nets. Furthermore, in some portions, the fibrils are thicker and seem to correspond to the grana of chloroplast in higher plants.

For the study on the structure of chromoplast, it is necessary to observe the structural development from the stage of proplastid. But the present report is the result of the study on only the mature stage of chromoplast. The relation between the lamellar structure and the fibrous structure in the chromoplast of the orange-fruit will be discussed in the further studies.

Summary

1. The ultrastructure of chromoplast seems to be fibrous in the orange-fruit. The fibrils are connected with each other. Some lipid globuli are found among the fibrils.
2. There are several thicker portions in the fibril. These portions correspond to "grana" of chloroplast in higher plants.
3. The ultrastructure of chromoplast is thought to be different depending upon species.

The present author expresses her cordial thanks to Dr. Yuasa, Professor of College of General Education, University of Tokyo, and Dr. Sato, University of Tokyo, for their advice and criticism in this study.

The help and encouragement of Mr. Nitta, University of Tokyo, are also gratefully acknowledged. The present author is likewise grateful to Mr. Sakata, the electron microscopical institute of University of Tokyo, and Mr. Inoue, Japan Electron Optic Laboratory Ltd., for valuable advice and kindly helping with the electron-microscopical technique.

References

- 1) Frey-Wyssling, A., and Kreutzer, E., *Planta* **51**: 104 (1958).
- 2) Steffen, K., and Walter, F., *Naturwiss.* **42**: 395 (1955).
- 3) —, and —, *Planta* **50**: 640 (1958).
- 4) Frey-Wyssling, A., and Kreutzer, E., *Jour. Ultrastr. Res.* **1**: 397 (1958).
- 5) Granick, S., *Handbuch der Pflanzenphysiologie*, Springer-Verlag **1**: 507 (1955).
- 6) Yuasa, A., Osumi, M., and Nakamura, H., *Sci. Pap. Coll. Gen. Edu. Univ. Tokyo* **7**: 2 (1957).

摘 要

大隅正子： 電子顕微鏡による有色体の研究 I. ミカンの有色体の電子顕微鏡的構造

1. ミカンの果実の有色体の構造は、電子顕微鏡でみると、せんい状に見える。せんいはからみあっている。せんいの間に、小粒が見え、脂質であると思われる。
2. せんいのところどころがふくらんでいて、高等植物の葉緑体のグラナにあたると思われる。
3. 有色体の電子顕微鏡的構造は、かならずしも一様でなく、植物の種類によってちがうようである。
(日本女子大学家政学部生物学教室)

Explanation of Plates

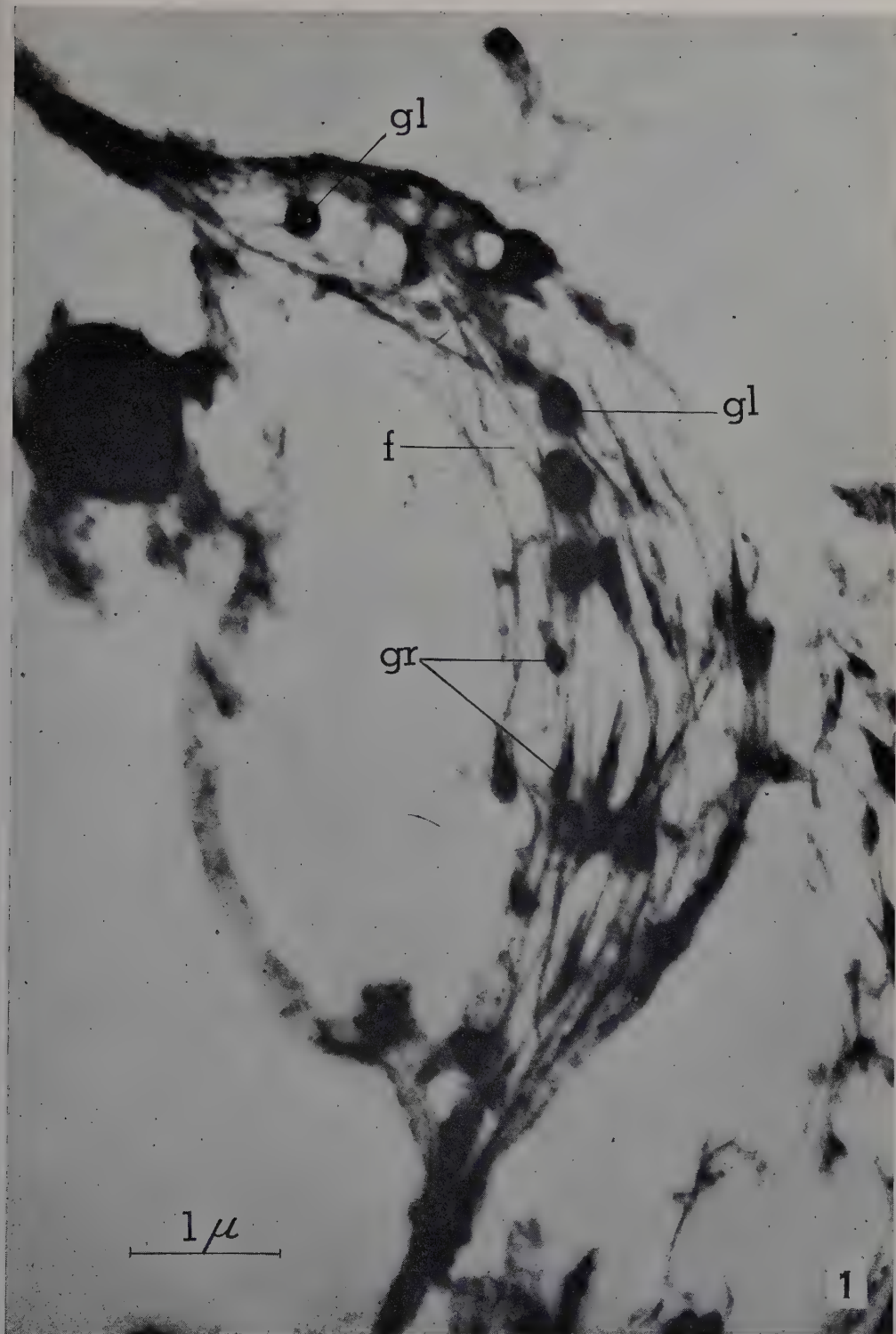
Plate I

Fig. 1. High magnification of electron-microphotograph of the chromoplast of *Citrus unshiu*. The chromoplast seems to be composed of many fine fibrils which are connected with each other and look like nets. In the fibril there are several thicker portions. These portions correspond to the grana of higher plants. Between the fibrils sometimes a few globuli exist. f, fibril; gr, granum; gl, globule. $\times 24000$.

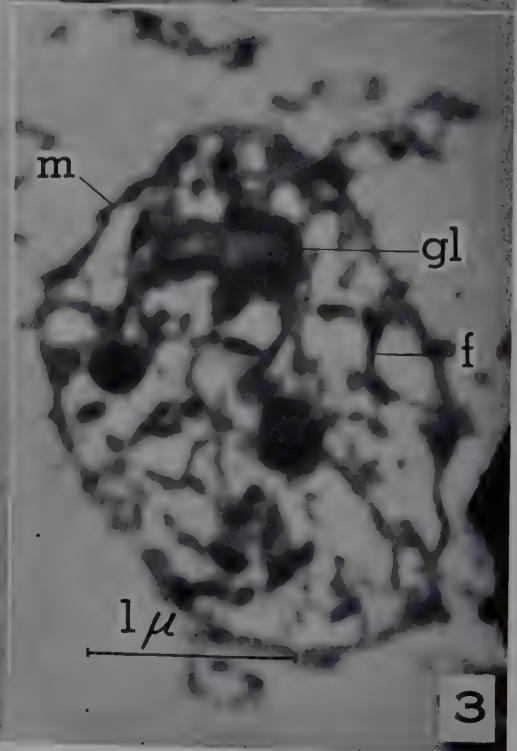
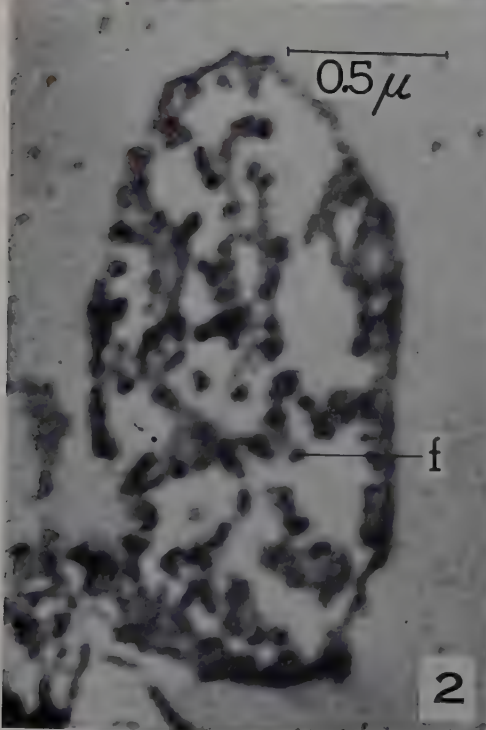
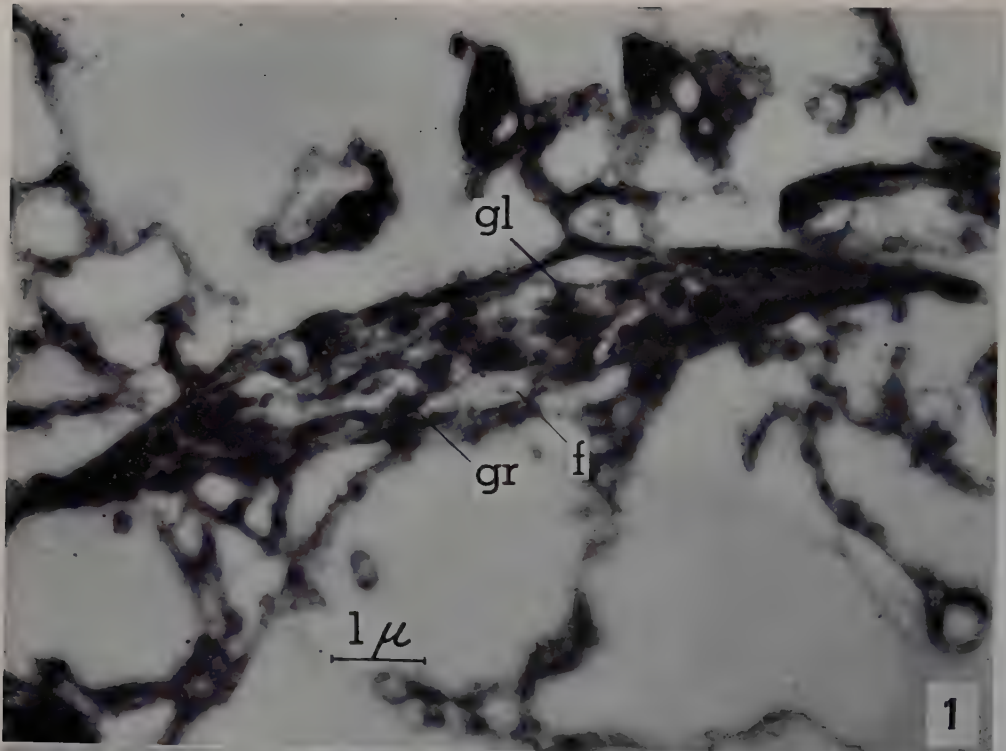
Plate II

Fig. 1. Electron-microphotograph of longitudinal section of the chromoplast in *C. unshiu*. f, fibril; gr, granum; gl, globule. $\times 12000$.

Figs. 2 and 3. Cross-sections of the chromoplast in *C. unshiu* by electron-microscope. The chromoplast seems to be covered with thin membrane. Inside the chromoplast-membrane, there are many small spots scattering which are the cross-sections of the fibrils (Fig. 2). In Fig. 3, owing to oblique section, thrile are shown the fibrils which look like nets. m, chromoplast membrane; f, fibril; gl, globule. Fig. 2, $\times 45000$; Fig. 3, $\times 30000$.



OSUMI, M.: Electron-microscopical Studies on Chromoplast
I. The Ultrastructure of Chromoplast in Orange



OSUMI, M.: Electron-microscopical Studies on Chromoplast
I. The Ultrastructure of Chromoplast in Orange

Water Uptake and Indoleacetic Acid Destruction of Cultured Bean Germ-Axes

by Mitsuo IZAWA*

Received July 29, 1960

Generally water uptake of plant tissues is said to be in an intimate correlation with their auxin content¹). Some authors are of opinion that the decrease in auxin destroying activity is a critical factor for cessation of water uptake or elongation of the tissues²).

In a previous paper³) it was reported that indoleacetic acid (IAA) remarkably affects the changes in fresh weight or water content of cultured germ-axes of a bean, *Vigna sesquipedalis*. Thus, the fresh weight of the germ-axes which increases gradually in the absence of IAA added, changes little in a IAA (1 μ g./ml.) containing medium for the first 2 days of culture, and then it begins to rise strikingly. That is to say, the exogenously supplied IAA apparently causes temporary inhibition of water uptake.

The present report concerns with this anomalous effect of IAA on water uptake of cultured bean germ-axes. It will be indicated that surplus auxin may inhibit not only water uptake but also IAA destroying activity of the tissues. The presence of a dialyzable substance in the tissues which can exert promotion at lower concentrations and inhibition at higher concentrations on IAA destroying system will also be demonstrated.

Materials and Methods

Seeds of *Vigna sesquipedalis* stored for about a year after harvest were used. The methods of isolation and culture of germ-axes were described elsewhere³).

Preparation of crude extract: Fifty to 60 germ-axes were harvested daily and homogenized with 30 ml. of 0.03 M phosphate buffer (pH 6.0) and sea sand in a porcelain mortar. The brei was centrifuged at 1,000 \times g (Kubota centrifuge, Model K-80) for 10 minutes to obtain *crude extract* of the tissues. Homogenization and centrifugation were conducted in a cold room at ca. 4°.

Dialysis: Thirty ml. of *crude extract* was placed in a seamless cellulose tube (A. H. Thomas Co., Philadelphia) and dialyzed against ca. 3 l. of distilled water for 24 hours with continuous stirring (a magnetic stirrer used) in the cold room to obtain *dialyzed extract*. The dialyzate solution, when its effect on IAA destroying activity was examined, was concentrated under reduced pressure at room temperature down to ca. 10 ml. (referred to as *dialyzate*).

Fractionation of crude extract: *Crude extract* (ca. 30 ml.) was centrifuged at 10,000 \times g (Servall centrifuge, Model SS-1) for 20 minutes to separate mitochondrial fraction from supernatant fraction. The sedimented pellet (mitochondrial fraction) was suspended in 6 ml. of 0.03 M phosphate buffer (pH 6.0) before use.

Measurement of IAA destroying activity: The reaction medium contained routinely 0.6 mg. IAA (free acid, Merck), 0.03 M phosphate buffer (pH 6.0) and designated

* Biological Institute, Faculty of Science, Nagoya University, Chikusa, Nagoya, Japan.

amount of *crude* or *dialyzed extract*, mitochondrial or supernatant fraction; total volume was 10 ml. When needed, MnCl_2 (10^{-4} – 10^{-7} M)*, hydrogen peroxide (10^{-4} M)**, 2,4-dichlorophenol (10^{-4} – 10^{-7} M), hydroquinone (10^{-5} – 10^{-7} M) or diluted *dialyzate* (1:10–1:1,000 by volume) was further added to the reaction medium. A shaking apparatus in a dark room was used; reaction temperature 30° . IAA destroying activity of extract or fraction was assayed as the amount of IAA ($\mu\text{g.}$) disappeared from the medium in one hour per protein-N (mg.). IAA was estimated colorimetrically as described by Galston and Dalberg²). Protein was precipitated with 10% trichloroacetic acid, and its nitrogen was estimated by Levy-Palmer's method.⁴)

Results

IAA destruction in crude extract.

IAA destroying ability of *crude extracts* prepared from germ-axes which were harvested daily is shown in Fig. 1. Two facts are noted in this figure: one is that in *control culture* (grown without exogenous IAA) the ability was the highest in 2 day-old culture and thereafter gradually declined until it was lost entirely on the 7th day. This result appears to be incompatible with Galston and Dalberg²) and Pilet

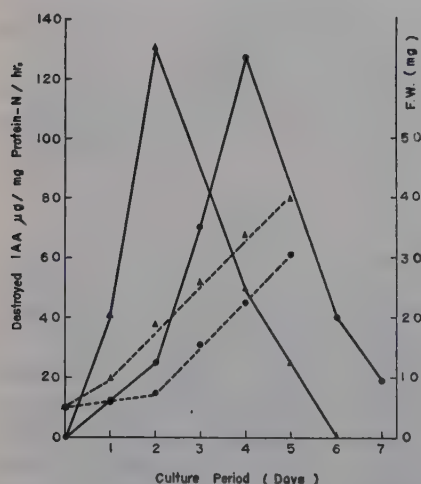


Fig. 1. IAA destruction in *crude extracts* and fresh weight of *control* and *IAA cultures* of various ages. Solid lines: IAA destruction, broken lines: fresh weight. Triangles: *control culture*, circles: *IAA culture*.

and Galston⁵) who indicated that IAA destroying activity of crude homogenates from pea epicotyl and *Lens* root increased with the age of the tissues. The other fact to be noted is that in *IAA culture* (grown with exogenous IAA) the ability was remarkably low for the initial 2 days. But after that time the activity showed a similar rise and fall to that observed in *control culture*. The pattern was not changed even if the culture medium (containing IAA) was renewed daily. As seen in the figure, this transitory depression of IAA destroying ability at the initial stage of *IAA culture* kept pace with the inhibition of water uptake of the tissues. The parallelism was undetectable in later period of culture when IAA destroying activity sharply declined independently of the water uptake. These facts also appear to be incompatible with the findings of other authors^{2,5}) that in pea epicotyl and *Lens* root the drop in IAA destroying activity accompanies the rise in water uptake and *vice versa*.

Effect of dialysis on crude extract.

In order to examine the mechanism of the inhibition of IAA destroying activity in the later culture period of both *control* and *IAA cultures* as well as in the early period of *IAA culture*, the effect of dialysis on *crude extracts* prepared from either

* Figures in parentheses indicate the final concentrations.

** Hydrogen peroxide of higher concentration ($>10^{-4}$ M) was found to destroy IAA non-enzymatically.

control or *IAA culture* of various ages was examined (Fig. 2). As shown clearly in the figure, the initial depression of the activity in *IAA culture* was nearly unaffected by dialysis, whereas the inhibition in the later stage of *control* and *IAA cultures* was removed entirely by dialysis. Moreover, the activity of *dialyzed extract* prepared from germ-axes in the later culture period was found to be hardly decreased by prolonged (2 to 3 day-long) dialysis. These facts suggest that the inhibition at the later stage of both cultures is due to some dialyzable substance(s), whereas the inhibition at the initial stage of *IAA culture* has nothing to do with such dialyzable inhibitor but likely is ascribed to the decrease in *IAA* destroying activity induced by exogenous *IAA*.

Effects of manganese, hydrogen peroxide, phenols and "dialyzed" on IAA destruction.

It is known that *IAA* destruction in plant tissues is an oxidative reaction catalyzed by peroxidase system, which is seriously affected by various substances such as Mn^{++} , H_2O_2 , monophenols and polyphenols^{6,7,8}).

The effect of the addition of 2, 4-dichlorophenol (DCP) on *crude* and *dialyzed extracts* prepared from 2 day-old *control culture* is seen in Fig. 3(a). DCP was found to act inhibitorily on *crude extracts* even at as low concentration as 10^{-7} M, while stimulatory on *dialyzed extracts* at 10^{-6} – 10^{-7} M. At higher concentrations ($\geq 10^{-5}$ M), however, DCP acted inhibitorily even on *dialyzed extracts*. Fig. 4(a) indicates that *IAA* destruction by *crude* and *dialyzed extracts* prepared from 5 day-old *control culture* is affected by DCP in a similar way to the case of the extracts from 2 day-old *control culture*. On the other hand, DCP had no effect of increasing but only that of decreasing the weak *IAA* destroying activity of either *crude* or *dialyzed extract* from 2 day-old *IAA culture* (Fig. 5(a)). Although no detailed description is given, DCP did not affect at all *crude* and *dialyzed extracts* prepared from 0 day-old germ-axes. The results obtained by other authors with other materials do not always coincide with the present data. For example, higher amounts of DCP (10^{-4} – 10^{-6} M) were reported to stimulate *IAA* destruction of crude homogenates prepared from pea epicotyls or wheat leaves^{2,6}). As generally accepted, DCP may act in *IAA* destroying system as a substitute for a naturally occurring phenolic cofactor^{9,10}), and these discrepancies may be explained in terms of the difference in concentration of endogenous phenolic cofactor. Thus bean *crude extract* may contain optimal amount of the cofactor, and exogenously supplied excess DCP would act only inhibitorily on *IAA* destroying system. On the other hand, in *dialyzed extract* the cofactor level may have been dropped sufficiently by dialysis, and now exogenous DCP can stimulate *IAA* destruction.

Hydroquinone (10^{-5} M) acted only inhibitorily on both *crude* and *dialyzed extracts* from *control* and *IAA cultures* of various ages (Figs. 3(b), 4(b) and 5(b)). This polyphenol, as a substrate for peroxidase, may compete with *IAA* for the enzyme action under consideration^{6,8}).

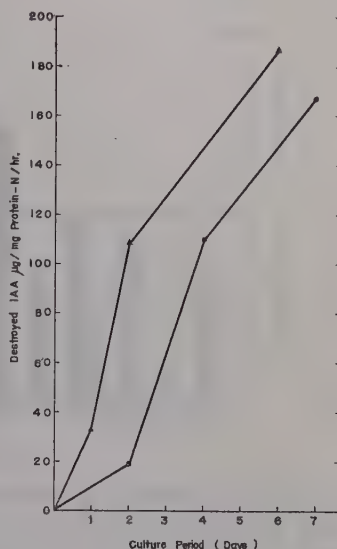


Fig. 2. *IAA* destruction in *dialyzed extracts* from *control* and *IAA cultures* of various ages. For symbols see the legend of Fig. 1.

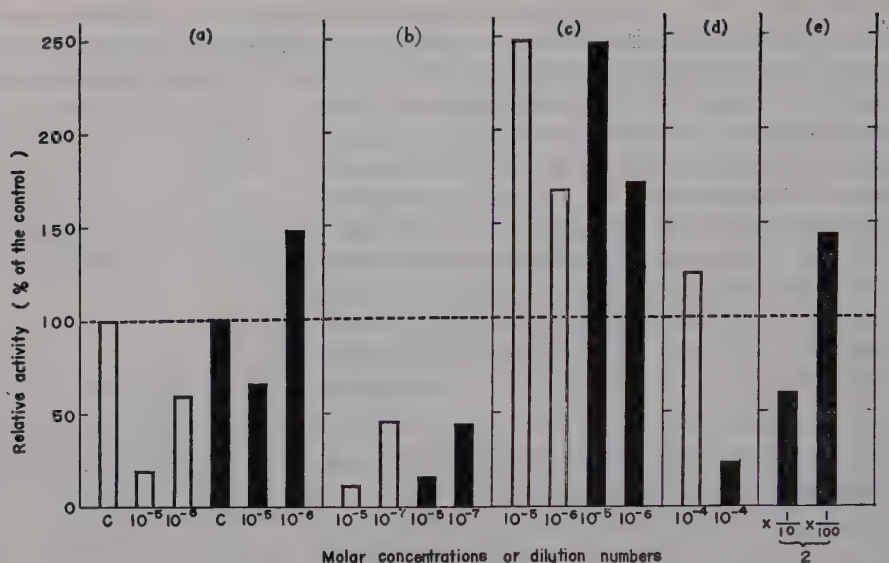


Fig. 3. Effects of 2, 4-dichlorophenol (DCP), hydroquinone, Mn^{++} , H_2O_2 and dialyzate on IAA destruction in crude (blank bars) and dialyzed (filled bars) extracts prepared from 2 day-old control culture. Figures shown on the abscissa are molar concentrations or dilution numbers (in (e)) of the additions.

- (a): control (C) and DCP effect, (b): hydroquinone effect,
 (c): Mn^{++} effect, (d): H_2O_2 effect,
 (e): dialyzate (prepared from 2 day-old control culture) effect.

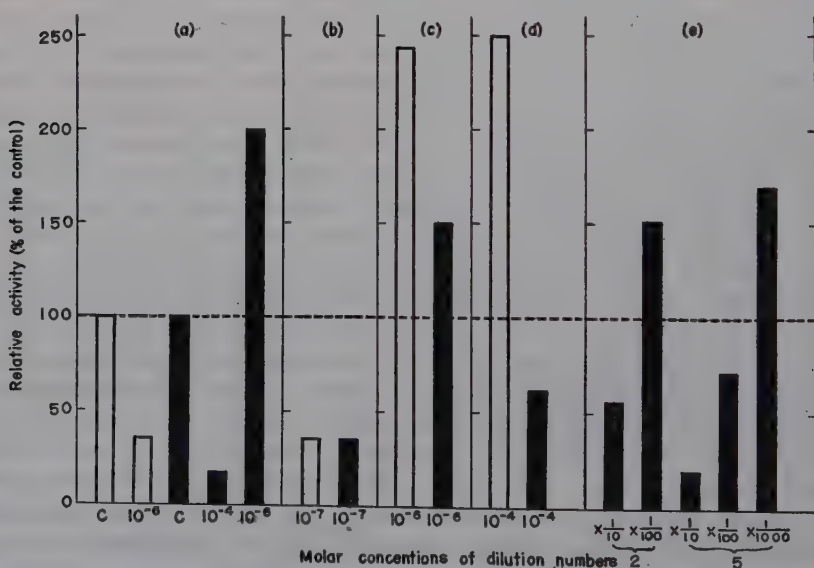


Fig. 4. Effects of DCP, hydroquinone, Mn^{++} , H_2O_2 and dialyzate on IAA destruction in crude and dialyzed extracts prepared from 5 day-old control culture. Dialyzates prepared from 2 and 5 day-old control cultures were examined respectively. For further details see the legend of Fig. 3.

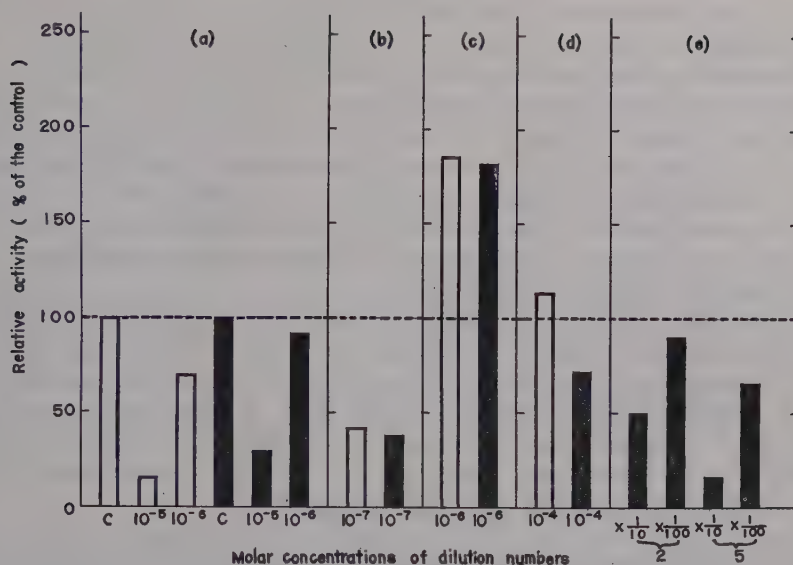


Fig. 5. Effects of DCP, hydroquinone, Mn^{++} , H_2O_2 and dialyzate on IAA destruction in crude and dialyzed extracts prepared from 2 day-old IAA culture. Dialyzates prepared from 2 and 5 day-old control cultures were examined respectively. For further details see the legend of Fig. 3.

Hydrogen peroxide acted promotively on crude extract from every culture. This may again relate to the participation of peroxidase in IAA destroying action. The reagent, however, acted rather inhibitorily on dialyzed extract regardless of culture conditions of the source materials (Figs. 3(d), 4(d) and 5(d)). The mechanism of this inhibition is not yet clear. It was also found that hydrogen peroxide could not evoke any IAA destroying activity in the extracts from 0 day-old germ-axes.

Table 1. Recovering effect of Mn^{++} on IAA destroying activity inhibited by DCP, hydroquinone or dialyzate.

Additions to reaction medium*	IAA destroyed ($\mu g./mg.$ protein-N/hr.)
none (control)	112
+DCP (10^{-5} M)	22
+DCP (10^{-5} M)+ Mn^{++} (10^{-5} M)	208
+hydroquinone (10^{-5} M)	15
+hydroquinone (10^{-5} M)+ Mn^{++} (10^{-5} M)	18
+dialyzate ($\times 1/10$)**	20
+dialyzate ($\times 1/10$)+ Mn^{++} (10^{-5} M)	204

* Reaction medium: 0.6 mg. IAA in 2 ml. of 0.03 M phosphate buffer (pH 6.0), 5 ml. of enzyme solution, crude extract prepared from 2 day-old control culture, made up to 10 ml. with the buffer.

** Dialyzate (see 'Dialysis' in Materials and Methods) diluted 10 times by volume with water was used.

Effect of manganese ions is also seen in Figs. 3(c), 4(c) and 5(c). $Mn^{++}(10^{-6} M)$ stimulated the rate of IAA destruction by both *crude* and *dialyzed extracts* prepared from germ-axes of various ages excepting 0 day-old ones. Mn^{++} added also completely reversed the inhibition caused by DCP (Table 1). But Mn^{++} could neither enhance the IAA destroying ability of the extracts from 0 day-old germ-axes nor remove the inhibition induced by exogenous hydroquinone (Table 1). Interaction of manganese and DCP on IAA destroying system was investigated by Hillman and Galston¹⁰⁾ in some details. They pointed to the possibility that Mn^{++} inhibits and promotes IAA destruction in tissue brei containing lower and higher levels of endogenous phenolic cofactors, respectively.

It was described above that *dialyzate* contained some factor(s) which could inhibit IAA destroying activity. Effect of *dialyzate* on *dialyzed extract* is illustrated in Figs. 3(e), 4(e) and 5(e). Figs. 4(e) and 5(e) suggest that the content of the factor(s) is higher in 5 day-old materials than in 2 day-old ones. It is noticeable that *dialyzates* from both 2 and 5 day-old tissues stimulated the destruction at a lower concentration and inhibited at a higher one. Inhibition by a higher concentration of added *dialyzates* was also diminished by $Mn^{++}(10^{-5} M)$ (Table 1).

Since any addition, i.e., Mn^{++} , H_2O_2 or DCP, could not evoke IAA destroying ability in 0 day-old extracts at all, the destroying system seems to be not yet functioning in germ-axes at the outset of culture.

Subcellular localization of IAA destruction system.

Subcellular localization of IAA destroying activity was investigated (Fig. 6). Mitochondrial (*Mt*) and supernatant (*Sp*) fractions from 2 day-old materials could

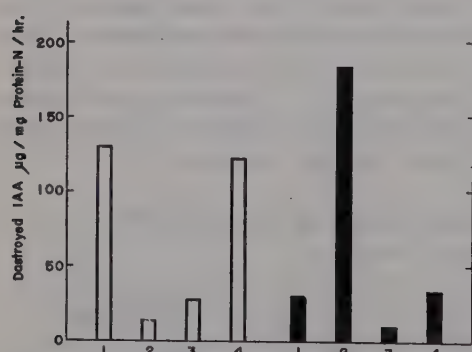


Fig. 6. IAA destruction by subcellular components prepared from 2 (blank bars) and 5 (filled bars) day-old control cultures.

- 1: crude extract, not fractionated,
- 2: mitochondrial fraction,
- 3: supernatant fraction,
- 4: mitochondrial+supernatant fractions.

hardly decompose IAA by themselves, but the recombination of these fractions evoked remarkable decomposition. Moreover, it was clearly demonstrated that the activity of *crude extract* from 5 day-old tissues was localized almost exclusively in *Mt* fraction, and some inhibiting substance(s) seems to exist in *Sp* fraction. This unknown substance(s), however, cannot be regarded as a simple inhibitor since the addition of small and large amounts of *Sp* from 5 day-old culture to *Mt* from 2 day-old culture stimulated and diminished the IAA destruction, respectively (Table 2). The results are very similar to those obtained in DCP experiments (Figs. 3(a) and 4(a)). As the amount of the active substance in *Sp* increases in some way with culture period, the action of *Sp* may be converted into inhibitory from promotive as was observed.

Table 2. Subcellular distribution of IAA destroying activity.

Fractions added to reaction medium*	IAA destroyed (μ g./mg. protein-N/hr.)
+Mt** (1 ml.)	10
+Sp*** (1 ml.)	7
+Sp (5 ml.)	12
+Mt(1 ml.)+Sp(1 ml.)	134
+Mt(1 ml.)+Sp(5 ml.)	37

* Reaction medium: 0.6 mg. IAA in 4 ml. of 0.03 M phosphate buffer (pH 6.0); total volume made up to 10 ml. with the buffer. For further explanations, see 'fractionation of *crude extract*' in the text.

** Mt fraction prepared from *crude extract* of 2 day-old control culture.

*** Sp fraction prepared from *crude extract* of 5 day-old control culture.

Discussion

In view of the effects of Mn^{++} , H_2O_2 , monophenol and polyphenol⁶⁻¹⁰), the bulk of IAA destruction by the extract of bean germ-axes appears to involve a peroxidative reaction.

Galston and Dalberg²) have found that in pea epicotyls IAA destroying activity is the lowest in the apical zone and increases basipetally along the epicotyl axis, and have suggested that the decrease in IAA level due to the increase of IAA oxidation capacity would be responsible for the cessation of elongation in the aged tissues. Similar result was also reported by Pilet and Galston⁵). They observed that in *Lens* root IAA destruction was the weakest at the meristematic zone and increased toward the maturation zone. In contrast, however, in the present materials, IAA destroying ability, as estimated similarly for *crude extract* to the above-cited authors, was elevated till the 3rd day of culture, and subsequently declined gradually to complete disappearance without any concomitant fall and rise in water uptake or elongation (Fig. 1). Another widely accepted view is that cell wall extensibility and/or water permeability of protoplasmic membrane are the essential factor for water uptake or elongation of plant tissues¹¹), and we have previously shown that disappearance of wall extensibility is the major cause of the cessation of water uptake in germinating bean hypocotyl⁴). At least in the present materials, therefore, IAA destroying activity may have no direct relation with water uptake or tissue elongation.

It is difficult, however, to answer whether IAA decomposing ability as assayed for extracts may reflect precisely the activity of the intact tissues. Separately we estimated the decrease in content of IAA added to a reaction medium in which isolated germ-axes were floated and shaken. Excepting the case where 0 day-old germ-axes were examined, definite loss of IAA from the medium was always found to take place. But it is still questionable if the activity *in vivo* was exactly measured here, since the decrease of IAA in the medium might simply be due to IAA absorption of the tissues.

The weak IAA destroying activity of *crude extracts* prepared from aged germ-axes was elevated strikingly with dialysis (Fig. 2). This suggests that some dialyzable inhibit or exists in *crude extract*. The effects of *dialyzate* and DCP on IAA destruction were found to be remarkably similar to each other; they stimulate the

destruction at lower concentrations and inhibit at higher ones, and the inhibition is removed by Mn^{++} . Hence a temporary conclusion may be that, in agreement with other authors^{7,9,10}), some phenolic compound is responsible for the stimulation and the inhibition of IAA destruction in bean germ-axis extract. But an alternative possibility is not entirely excluded that the inhibition and the stimulation are brought forth by different yet unidentified substances, respectively¹²). An interesting finding is that in aged germ-axes IAA destroying activity (peroxidase activity?) and the active substance(s) in question were shown to localize in mitochondrial and $10,000\times g$ supernatant fractions, respectively, while in younger cells (2 day-old) the activity was manifested only when both fractions were combined. In the present experiments it was also found that IAA destroying ability in *dialyzed extract* prepared from germ-axes at later culture period was not removed with prolonged dialysis. On the other hand, a purified peroxidase preparation has been shown to be still in combination with some cofactor⁸). Thus, the exclusive localization of the activity in *Mt* fraction in aged cells may be ascribed to the binding of the active substance (cofactor) from *Sp* fraction with the enzyme protein moiety in *Mt* fraction.

In *IAA culture* only weak IAA destruction was exhibited for the initial 2 days. This weak activity could not be promoted by dialysis, and likely related to the inhibitory action of auxin added in excess. Separately it has been revealed that the concentration of ether-extractable auxin is the highest in 0 day-old germ-axes as far as the germination stage is concerned. Thus the exogenously supplied IAA may readily rise the auxin level of the tissues over a threshold so that their IAA destroying activity would be depressed. During the initial 2 days of culture this surplus IAA may be destroyed slowly with this remaining weak activity until the IAA concentration falls down below the threshold level, and this would allow more active IAA destruction and normal water uptake to proceed.

Summary

1. IAA destruction by extracts prepared from bean germ-axes cultured in a medium with or without IAA added was investigated.
2. In *control culture* (grown without exogenous IAA) IAA destroying activity as measured for *crude extract* was at its maximum in the 2 day-old materials and decreased henceforth. The declined activity was increased considerably with dialysis of the extract.
3. In *IAA culture* (grown with exogenous IAA, $1\mu g./ml.$) IAA destroying activity changed as in *control culture* but with a definite lag period of 2 days where only weak activity was manifested. This initial depressed activity was not promoted by dialysis.
4. Addition of a small amount of *dialyzate* from either 2 or 5 day-old tissues to *dialyzed extract* stimulated IAA destruction, whereas the addition of a large amount inhibited it. This inhibition was reversed with Mn^{++} .
5. Combined with the results obtained especially from the examinations on the effects of H_2O_2 and phenols, the involvement of peroxidase reaction in IAA destruction and the presence of an endogenous phenolic substance affecting IAA destroying activity was assumed.

The author wishes to express his sincere gratitudes to Dr. Y. Oota for his kind guidance and advice.

References

- 1) Leopold, A. C., Auxins and Plant Growth, Berkeley (1955).
- 2) Galston, A. W., and Dalberg, L. Y., Amer. Jour. Bot. **41**: 373 (1954).
- 3) Izawa, M., Bot. Mag. Tokyo **74**: 98 (1961).
- 4) —, Jap. Jour. Bot. **16**: 135 (1958).
- 5) Pilet, P. E., and Galston, A. W., Physiol. Plantarum **8**: 888 (1955).
- 6) Ray, P. M., Ann. Rev. Pl. Physiol. **9**: 81 (1958).
- 7) Kenten, R. H., Biochem. Jour. **59**: 110 (1955).
- 8) Yamazaki, I., Proc. Int. Sym. Enz. Chem. **2**: 224 (1958).
- 9) Goldacre, P. L., Galston, A. W., and Weintraub, R. L., Arch. Biochem. Biophys. **43**: 358 (1953).
- 10) Hillman, W. S., and Galston, A. W., Physiol. Plantarum **9**: 230 (1956).
- 11) Söding, H., Wuchsstofflehre, Stuttgart (1952).
- 12) Galston, A. W., in Photoperiodism and Related Phenomena in Plants and Animals, Washington (1959).

摘 要

井沢三生: ミトリササゲの培養胚における吸水とインドール酢酸分解

IAA (1 $\mu\text{g./ml.}$) 添加および無添加 (対照) それぞれの条件下で組織培養されたミトリササゲ胚の吸水能および IAA 分解能は日とともに特色ある変化を示す。対照培養の吸水能は培養期間を通じて不変だが、IAA 分解能はゼロから出発して 2 日目に極大値に達し、以後降下して 6 日目にはふたたびゼロになる。IAA 培養では、吸水能、分解能ともに最初の 2 日間顕著な lag を示すが、以後対照培養に見られるのと同様に化する。

培養後期の IAA 分解能低下の原因は、組織の可溶性分画中に、ある種のモノフェノールが蓄積することにあるらしい。培養初期の胚では、この物質は、その組織内濃度が低いため、むしろ逆に IAA 分解促進作用を営んでいる可能性が大きい。

IAA 培養初期の低 IAA 分解能は、外部からの IAA 供給に基づく組織内 IAA 濃度上昇にその原因があると考えられる。

培養胚の営む IAA 分解はパーオキシダーゼ作用を含むであろう。(名古屋大学理学部生物学教室)

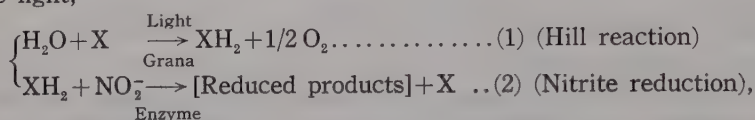
Photosynthetic Nitrite Reductase I. Partial Purification and Properties of the Enzyme from Spinach Leaves*

by Hiroshi HUZISIGE** and Kimiyuki SATOH**

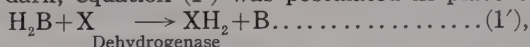
Received September 27, 1960

In a previous paper¹⁾ it was shown that the intact cells of *Euglena* showed a noticeable activity of nitrite reduction, and this reaction was accelerated both by exposure to light and by the addition of some suitable hydrogen donors. The authors suggested a possible mechanism for nitrite reduction as follows, based upon the experimental evidence reported in that paper:

in the light,



in the dark, equation (1') was postulated in place of equation (1),



where X and H₂B represent the hydrogen transporting system and the hydrogen donor, respectively.

Attempts to demonstrate nitrite reduction using the cell-free system of *Euglena*, in order to provide further experimental evidence for this reaction scheme, have achieved little success, because of technical difficulties in gathering materials in large quantities, and because of the slight accelerating effect of light, although a definite photochemical nitrite reduction was always observed.

The present paper is concerned with the isolation from spinach leaves of a soluble enzyme preparation, which is required in addition to grana for the photochemical nitrite reduction. The summarized results of kinetic studies on the reaction in question are also described. Further experiments on the chemical pathway of this reaction system and further purification of the enzyme preparation will be published elsewhere.

Materials and Methods

(1) *Preparation of grana*: Fresh leaves of spinach (ca. 800 g. for each preparation) were freed from veins and macerated in small volumes of the extracting solution (ca. 100 ml.) in a Waring blender for 3 minutes at top speed. The homogenate was squeezed through cheesecloth to remove coarse material and the filtrate was centrifuged for 3 minutes at 800×g. The supernatant fluid (referred to as G₁) was again centrifuged for 20 minutes at 25,000×g; the residue was suspended in the extracting solution (suspension G₂), and the greenish supernatant fluid (crude enzyme extract: E₀)*** was subjected to a further purification procedure for enzyme preparation. The sediment obtained by centrifuging the G₂-suspension was resuspended in smaller volume of the same extracting solution and used as the final material of grana preparation (this is referred to as G₃). On microscopic examination, the grana in the G₃-suspension were

* Preliminary report upon this work was presented at the 24th annual meeting of the Botanical Society of Japan (1959, Sendai).

** Department of Biology, Faculty of Science, Okayama University, Okayama, Japan.

*** This fraction still showed a remarkable activity of the Hill reaction.

found to be of a uniform size.

As the extracting solution, M/90 phosphate buffer (pH 7.0) containing 0.4 M sucrose was used in the preliminary experiments, but Tris-HCl buffer (pH 7.1) was found to be more suitable for obtaining grana preparations of high activity, and this buffer solution was used in all the experiments described in this paper.

The whole procedure of extraction was carried out at 0~5°C, and the grana preparation (G_3) was prepared just prior to use, because of its instability (See p. 181).

(2) *Preparation of partially purified enzyme*: Crude enzyme extracts (E_0) were purified by acetone fractionation. Acetone, previously cooled to -5°, was slowly added to the crude enzyme extract with mechanical stirring to a final concentration of 50 per cent, and after standing for 10 minutes, the mixture was centrifuged for 10 minutes at $800 \times g$. Cold acetone was again added to the greenish-brown supernatant solution to a final concentration of 80 per cent. After several minutes, the greater part of the supernatant fluid was decanted, and the precipitate was collected by centrifugation for 10 minutes at $1,000 \times g$. The partially purified enzyme thus obtained was dissolved in a small volume of buffer solution (clear, chocolate-coloured) and used in most of the following reconstruction experiments, and this is referred to as preparation E_4 .

Further purification of the enzyme has been accomplished in our laboratory by the use of active alumina adsorption and diethyl-aminoethyl-cellulose chromatography, the details of which will be described in the following paper.

(3) *Assaying procedure*: The method of measuring the nitrite-reducing activity was essentially the same as that described in the previous paper¹⁾, and only a brief outline will be given here. Thunberg tubes illuminated under continuous shaking in a thermostat (25°) were used as reaction vessels. Since there was no difference in nitrite reduction under anaerobic, and aerobic conditions, the gas space remained unchanged. The light intensity at the surface of the reaction vessel was uniformly about 20,000~25,000 lux. Dark reaction was carried out in reaction vessels covered with aluminium foil. The composition of the standard reaction mixture was as follows: 1.5 ml. of grana suspension, 1.0 ml. of enzyme solution, 1.5 ml. of buffer solution, and 1.0 ml. of 10^{-3} M/l. NaNO_2 . At various intervals during the reaction period, the reaction vessels were removed from the thermostat one by one, and the reaction was instantaneously stopped by the addition of a saturated solution of uranyl acetate. After bringing the mixture up to 12.5 ml. with distilled water and centrifuging at $25,000 \times g$. for 5 minutes, the supernatant fluid was assayed for nitrite. The estimation of nitrite concentration was essentially the same as that described by Novak and Wilson²⁾. To 2.5 ml. of the supernatant fluid, 7.5 ml. of distilled water and 1.0 ml. of Griess-Ilosvey's reagent were added, and after shaking for 15-20 minutes, the red colour formed was measured spectrophotometrically at $530 \text{ m}\mu$. The protein contents of the enzyme preparations were determined by the ultraviolet absorption at 260 and $280 \text{ m}\mu$ after Kalcker's method³⁾, and the method of MacKinney⁴⁾ was used for chlorophyll determination.

Results

(1) *Nitrite reduction by homogenate of green leaves*: As an initial step to the analysis of nitrite reduction by a cell-free system, the first centrifugal fraction (G_1) was subjected to an examination of the activity of the reaction in question. The results obtained both in the light and in the dark are given in Fig. 1. As will be seen from the figure, light has a pronounced effect on nitrite reduction, the ratio of the reaction

rate in the light to that in the dark being about 9:1, a ratio greater than that obtained with *Euglena* cells¹). Although not indicated in the figure, an exact proportionality between the concentration of the homogenate used and the initial velocity was observed over a wide range.

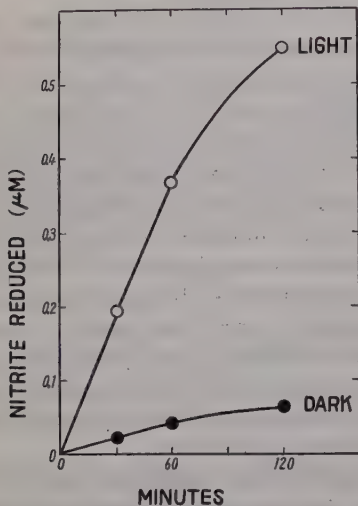


Fig. 1. Nitrite reduction by homogenate of spinach leaves. The reaction mixture consisted of homogenate (G_1) 4.0 ml. and Na-nitrite (10^{-3} M/l.) 1.0 ml. Light reaction was carried out under an intensity of 20,000–25,000 lux; the dark reaction vessel was shielded with aluminium foil. All reaction vessels were incubated in a thermostat at 25°.

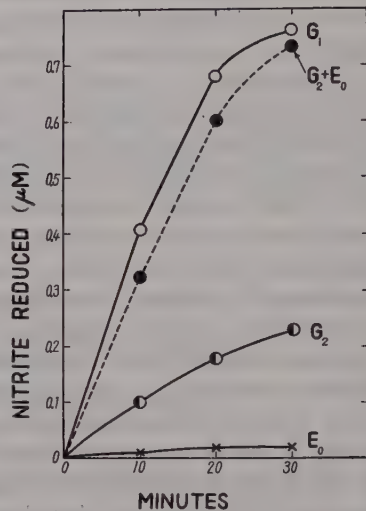


Fig. 2. Fractionation of homogenate (G_1) into grana (G_2) and enzyme (E_0). The chlorophyll content in G_2 was made equal to that in G_1 . Detailed explanation in text.

(2) *Fractionation of homogenate into grana and enzyme:* The activities of photochemical nitrite reduction measured with three centrifugal fractions, i.e. G_1 , G_2 and E_0 , were compared. As shown in Fig. 2, the activity of homogenate G_1 was found to be markedly reduced by separation into grana G_2 and crude enzyme E_0 . It was interesting, however, that the diminished activity in G_2 was restored to a rate comparable to that obtained with G_1 , provided that crude enzyme fraction E_0 was added to the reaction mixture. To ascertain the above-mentioned relationship, we carried out a more elaborated reconstruction experiment, using the partially purified enzyme preparation (E_4) and the three-times washed grana preparation (G_3). The results are summarized in Table 1. It will be seen from this table that a noticeable rate of nitrite reduction is obtained only when the reaction mixture was illuminated in the presence of the two components (E_4 and G_3), while illuminated grana or enzyme alone showed a negligible rate of reduction. The enhancement of activity on mixing G_3 and E_4 was scarcely observed when one of these fractions had been boiled.

In view of the experimental evidence obtained above, we may infer that some reducing substances produced photochemically as a result of the Hill reaction are transferred to the nitrite reducing system in the enzyme preparation, thus leading to the reduction of nitrite in the light. The activity of this enzyme is assumed to

depend greatly on the photochemical activity of the grana preparation, although the enzyme preparation showed a slight activity of nitrite reduction also in the dark, provided that a suitable amount of hydrogen donor (e.g., malate) was added. It is, therefore, tentatively proposed that the enzyme should be named "photosynthetic nitrite reductase".

Table 1. Typical result of reconstruction experiment. The following abbreviations are employed: E₄, partially purified enzyme preparation; G₃, three-times washed grana preparation; BE₄, BG₃, the samples (E₄ or G₃) treated at 70° for 3 min. The amount of nitrite reduced is expressed as the quantity of nitrite reduced in 5 min.

Components of reaction mixture	Light condition	Nitrite reduced (μ M)
1. NaNO ₂ +G ₃	Light	0.02
2. NaNO ₂ +E ₄	Light	0
3. NaNO ₂ +G ₃ +E ₄	Light	0.68
4. NaNO ₂ +G ₃ +E ₄	Dark	0
5. NaNO ₂ +BG ₃ +E ₄	Light	0
6. NaNO ₂ +G ₃ +BE ₄	Light	0.01

(3) *Stability of nitrite reduction system*: The stability of the nitrite-reduction system was investigated by comparing the following four combinations; E-G, E-OG,

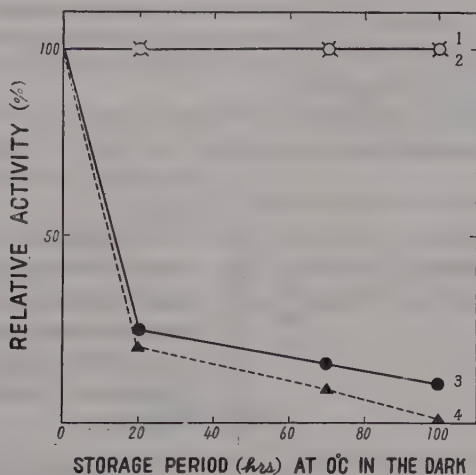


Fig. 3. Stability of nitrite reduction system. The rates of photochemical nitrite reduction by respective combinations which were measured after standing at 0°C for varying periods in the dark and expressed as the amount of nitrite reduced ($\times 10^{-7}$ M) in 10 min. were plotted as percentage of the rate of original activity measured with E₄-G₃-system at just after their preparation. 1, ○—○, E-G; 2, ×—×, OE-G; 3, ●—●, E-OG; 4, ▲—▲, OE-OG. The reaction mixture consisted of enzyme (20 mg. protein/ml.) 1.0 ml., grana (1.8 mg. chlorophyll/ml.) 1.0 ml., Tris-HCl buffer (0.05 M, pH 7.1) 2.0 ml. and NaNO₂ (2×10^{-3} M/l.) 1.0 ml. Temperature, 25°; light intensity, 20,000–25,000 lux.

OE-G and OE-OG, where E and G represent the freshly prepared enzyme preparation (E_4) and grana (G_3), respectively, and OE and OG represent the similar preparations aged by storing in an ice-box for varying lengths of time prior to the test. As will be seen from Fig. 3, the observed decline in capacity for photochemical nitrite reduction is mainly ascribable to the deterioration of the grana.

(4) *Kinetic studies on photochemical nitrite reduction:*

(a) *Effect of enzyme concentration:* The relationship between the initial velocity of nitrite reduction and the enzyme concentration was investigated, and the results are shown in Fig. 4. As can be seen, a linear relationship exists between reaction rate and enzyme concentration within the limit of 10 mg. enzyme-protein per vessel and at higher enzyme concentrations the reaction rate becomes gradually saturated to show a plateau in the curve. From these findings, enzyme concentrations of 20–30 mg. protein per vessel were therefore employed in the following experiments, except when the concentration of the enzyme was reduced to make it the rate-determining factor.

(b) *Effect of grana concentration:* The initial velocity of the reaction in question increased with the increase in grana concentration up to 2 mg. chlorophyll per vessel, higher concentrations of grana above 3 to 4 mg. chlorophyll per vessel rather retarding reaction rate, as shown in Fig. 5. This may be interpreted

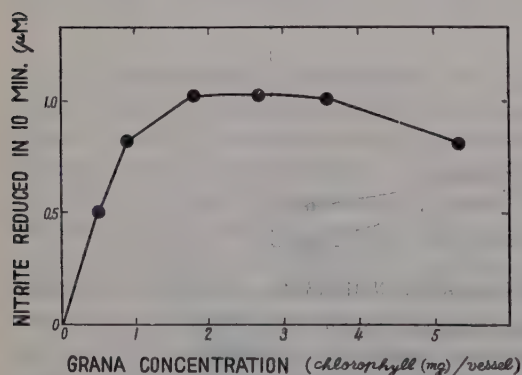


Fig. 5. Effect of grana concentration on photochemical nitrite reduction. Enzyme (E_4), 31.0 mg. protein per vessel, was used. Light intensity, 20,000–25,000 lux; 25°.

as to be due to the mutual shading effect.

(c) *Effect of substrate concentration:* The (apparent) Michaelis constant with respect to the reaction in question was computed from the data of reaction rate *versus* substrate concentration, according to the following equation;

$$1/v = K_m/V(1/[S]) + 1/V.$$

From $1/v \cdot 1/[S]$ -curve, it was possible to read off the value for K_m as 3×10^{-4} M/l.

(d) *Effect of pH:* As indicated in Fig. 6, the pH range of this reaction was found to be 5.5–7.0 with a maximum at pH 7.0. The increase in activity in the acidic region below pH 4.0 is probably due to the non-enzymatic disappearance of nitrite under the acidic condition.

(e) *Effect of buffer:* Three buffer solutions, i.e. Tris-HCl buffer (M/5 Tris-(hydroxymethyl)-aminomethane, M/10 HCl), McIlvaine's citrate buffer (M/5 Na_2HPO_4 ,

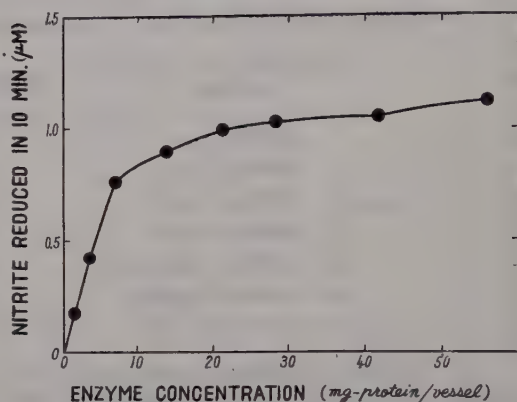


Fig. 4. Effect of enzyme concentration on photochemical nitrite reduction. Grana (G_3), 2 mg. chlorophyll per vessel, was used. Light intensity, 20,000–25,000 lux; 25°.

M/10 citric acid) and phosphate buffer (M/15 Na_2HPO_4 , M/15 KH_2PO_4), were used to test the effect of the nature of the buffering system upon the reaction rate of nitrite reduction at pH 7.1. As shown in Fig. 7, Tris-HCl buffer was found to be the most

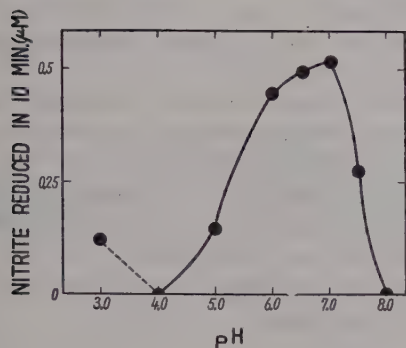


Fig. 6. Effect of pH on photochemical nitrite reduction. McIlvaine's citrate buffer (pH 3.0~8.0) were used. Enzyme (E_4), 21.5 mg.-protein per vessel, and grana (G_8) 2.6 mg.-chlorophyll per vessel, were used, respectively. Light intensity, 20,000—25,000 lux; 25°.

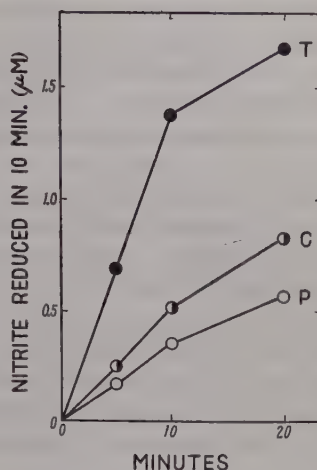


Fig. 7. Effect of the composition of buffer solutions on photochemical nitrite reduction. The following three buffer solutions of pH 7.1 were used: T, ●, Tris-HCl buffer; C, ●, McIlvaine's citrate buffer; P, ○, phosphate buffer. Enzyme (E_4), 21.5 mg.-protein per vessel, and grana (G_8), 2.6 mg.-chlorophyll per vessel, were used. Light intensity, 20,000—25,000 lux; 25°.

suitable for this reaction. It should be noted that no such marked response to phosphate as reported in the previous paper¹) was observed in this case, although the reason for this lack of phosphate effect in the nitrite reduction in this case of a cell-free system awaits further elucidation.

Discussion

A more remarkable activity of photochemical nitrite reduction than that of *Euglena* cells reported in the previous paper¹) was observed with the homogenates of spinach leaves. The marked difference between spinach extracts and *Euglena* cells may indicate a difference between the photochemical activities of strictly autotrophic plants (e.g. *Spinacia*) and facultatively autotrophic microorganisms (e.g. *Euglena*), and it may be supposed that autotrophic plants carry on many light-dependent metabolic activities besides photosynthesis.

Fractionation of the photochemical nitrite reduction system of spinach leaves into a grana system and an enzyme system has been achieved in this study. Using these two components, i.e. enzyme preparation ("photosynthetic nitrite reductase") and grana preparation of spinach, we could clearly demonstrate that the photochemical nitrite reduction results from their coagency. A marked nitrite reduction was ob-

served only when the two components were added together and illumination was also provided, whereas grana or enzyme alone showed a negligible activity of nitrite reduction both in the light and in the dark. It is evident from the data presented above that, grana is endowed with a thus far undiscovered capacity for a new type of chloroplast reaction, i.e. photosynthetic nitrite reduction, besides the Hill reaction, photosynthetic phosphorylation, etc., provided that a newly isolated leaf enzyme is added. Accordingly, the latter factor was named "photosynthetic nitrite reductase".

We have presented¹⁾ a reaction scheme for the reduction of nitrite, in which some reducing substances produced either by the Hill reaction or by the dehydrogenation of hydrogen donor may play an important role, and we could bring further evidence in this report as to the enzymatic nature of the process.

Despite several similarities in the methods of preparation and the properties of our "photosynthetic nitrite reductase" and those of "photosynthetic pyridine nucleotide reductase" of San Pietro and Lang^{6,7)}, these two enzymes differ in that the activity of the former is inhibited rather than accelerated by the addition of TPN*. Another point of interest in this connection is the elucidation of the mechanism by which the reducing substance is transferred from the chloroplast to the enzyme system. Further studies are needed to elucidate the functional role that the enzyme plays in the mechanism of photochemical nitrite reduction.

Summary

1. The homogenates of spinach leaves show a remarkable activity of nitrite reduction, and this reaction was found to be accelerated by exposure to light. The ratio of the reaction velocity in the light to that in the dark is about 9:1; a ratio greater than that obtained with *Euglena* cells (2:1).

2. The separation of the homogenate into grana and enzyme was achieved, the reduction of nitrite occurring only when these two components were added together, and illumination was also provided, whereas either preparation alone showed a negligible rate of nitrite reduction. This enzyme was designated tentatively as "photosynthetic nitrite reductase", and partially purified by acetone fractionation from crude extracts of spinach leaves.

3. The biochemical properties of this enzyme have been examined by investigating the effects of enzyme concentration, grana concentration, substrate concentration, pH and the composition of buffer solutions upon the rate of photochemical nitrite reduction.

4. Additional evidence for the reaction scheme proposed in the preceding paper was discussed.

The authors wish to express their thanks to Mr. Tadao ARABORI, who collaborated in some of the experimental work. Thanks are also due to Dr. McCrimmon for her kindness in reading the original manuscript, and to Dr. A. TAKAMIYA of Tokyo University for his criticism and encouragement.

References

- 1) Huzisige, H., and Satoh, K., Biol. J. Okayama Univ. **6**: 1 (1960). 2) Novak, R., and Wilson, P. W., J. Bact. **55**: 517 (1948). 3) Kalcker, H., J. Biol. Chem. **167**: 461 (1947). 4) MacKinney,

* Unpublished data to be reported elsewhere.

G., J. Biol. Chem. **140**: 315 (1941). 5) Evans, H. J., and Nason, A., Plant Physiol. **28**: 233 (1953). 6) San Pietro, A., and Lang, H. M., Science **124**: 118 (1956). 7) San Pietro, A., and Lang, H. M., J. Biol. Chem. **231**: 211 (1958).

摘 要

藤茂宏・佐藤公行： 光化学的亜硝酸還元酵素（第1報）ホウレンソウから抽出した酵素の純化およびその性質

- 1) ホウレンソウの葉のホモジェネートに強力な光化学的亜硝酸還元系の存在することを証明した。これは新しい型の葉緑体反応である。
- 2) ホモジェネートからグラナ標品 (G) と酵素標品 (E: Photosynthetic nitrite reductase とよぶ) とをそれぞれ分離し、精製したものをを用いて再構成実験を行なった。G または E 単独では亜硝酸還元は起こらないが、G と E とを共存せしめて光を与えると、顕著な亜硝酸還元反応が起る。
- 3) E と G とよりなる光化学的亜硝酸還元系におよぼす酵素濃度・グラナ濃度・基質濃度・pH・緩衝溶液の組成の影響をしらべ、この系の生化学的諸性質を明らかにした。
- 4) 前報で提出した機作模式の妥当性に関して論議した。(岡山大学理学部生物学教室)

Concerning the Anthocyanins of Two Garden Varieties of *Tulipa Gesneriana*

by Mannen SHIBATA and Emi SAKAI*

Received October 1, 1960

In the preceding papers of this series, the structure and properties of two anthocyanin chlorides tulipanin and keracyanin isolated in crystalline state from tulip flowers have been described^{1,2)}.

On the other hand, paper-chromatographic survey of anthocyanins recently carried out in our laboratory on 107 garden varieties of tulip has disclosed that the flowers ranging from red to dark purple contain 2-4 anthocyanins in general, and that six kinds of anthocyanin may be enumerated throughout the varieties examined^{3,4)}.

The present paper includes further examples for the crystallization of two anthocyanins from tulip varieties. The perianths of two garden varieties, "The Bishop" (Darwin strain) and "Parrot Pierson" (Parrot strain), were used as material. The extraction and crystallization of the pigments were carried out as usual. The yield of crystalline chloride was exceedingly poor; namely, 0.012 % for "The Bishop" and 0.006 % for "Parrot Pierson" on a fresh weight basis. The sugar moieties in these anthocyanins were found to be glucose and rhamnose. The anthocyanidins were identified as delphinidin and cyanidin, respectively. Paper-chromatographic as well as spectrophotometric examinations and elementary analyses have shown that of these two anthocyanins, the one was nothing but tulipanin (delphinidin-3-glucorhamnoside) and the other was keracyanin (cyanidin-3-glucorhamnoside).

Experimental

A. "The Bishop"-perianths

Isolation of tulipanin as chloride. Fresh perianths (ca. 28 kg.) were immersed in cold methanol (10 l.) containing 1 % hydrochloric acid. After standing for 2 days, the filtered red extract (9 l.) was added with a saturated methanolic solution of basic lead acetate (ca. 2 l.), under continuous agitation. The blue lead salt precipitated was collected by suction and washed with water and methanol. The dried lead salt (ca. 370 g.) was finely powdered and converted into chloride by dissolution in 5 % methanolic hydrochloric acid (ca. 2 l.). The dark red filtrate was concentrated to 2/5 volume *in vacuo* below 35°, and stored in a refrigerator to make further impurities separate out. After filtration, the anthocyanin in the filtrate was precipitated with ether (7 vol.). The dark red resinous precipitate obtained was dissolved in a small amount of 1 % ethanolic hydrochloric acid and precipitated again with ether (5 vol.). Amorphous precipitate formed was then dissolved in a small quantity of ethanol containing 1 % hydrochloric acid and filtered, and 1/2 vol. of 5 % ethanolic hydrochloric acid was added to it. Soon, purplish red-brown needles commenced to separate in sea-urchin-like cluster. Yield 3.6838 g. Crude crystals were recrystallized from warm water containing 5 % ethanolic hydrochloric acid. The yield of pure crystals corresponded to about 0.012 % of the fresh weight of perianths used.

* Laboratory of Plant Physiology, Biological Institute, Toyama University, Toyama, Japan.

Anthocyanin chloride (tulipanin). The "Bishop"-anthocyanin crystallized in purplish red-brown needles, which decomposed at 178° , either alone or on admixture with authentic specimen of tulipanin. The distribution number between *iso*-amyl alcohol and 0.5 % hydrochloric acid was about 9.3. On irrigation with *n*-butanol/conc. hydrochloric acid/water (7:2:5, v/v), one single spot was obtained on the chromatogram showing $R_f=0.38$. This was also the case on co-chromatography. The light absorption (Fig. 1), solubility in usual solvents and colour reactions were quite identical with those of tulipanin. Found: C 49.99; H 4.74. Calc. for $C_{27}H_{31}O_{16}Cl$: C 50.12; H 4.83. Water of crystallization: Found: H_2O 10.22. Calc. for $C_{27}H_{31}O_{16}Cl \cdot 4 H_2O$: H_2O 10.02.

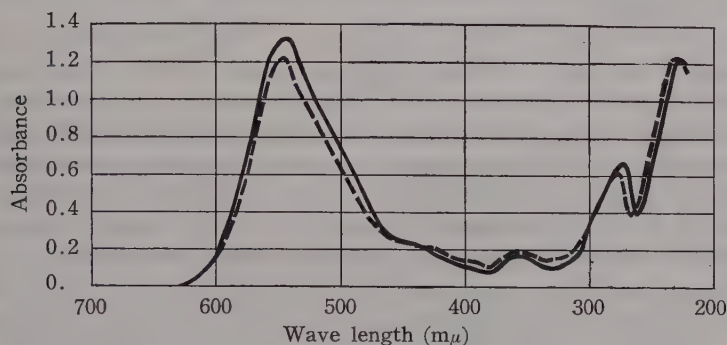


Fig. 1. Absorption spectra of "The Bishop"-anthocyanin and tulipanin.

— "The Bishop"-anthocyanin, 4/10,000 mol in 60 % EtOH (HCl-conc. 0.1 %)

----- Tulipanin (from "Queen of Night"), 4/10,000 mol in 60 % EtOH (HCl-conc. 0.1 %)

Hydrolysis. The purified glycoside (1 g.) was dissolved in water (80 mL.), and after addition of conc. hydrochloric acid (50 mL., d 1.18), boiled for 3 min. and stood in a refrigerator for 24 hrs. The aglycone separated completely in blackish mass having greenish luster (0.47 g.). The acidic mother liquor was shaken with ether, in which no trace of organic acids could be detected. The acidic solution was then shaken with *iso*-amyl alcohol to remove a last trace of aglycone, and subjected to the examination of sugar components. Finally, the sugars were identified as glucose and rhamnose, respectively, by colour reactions, paper-chromatographic test as well as by osazone formation.

Aglycone (delphinidin chloride). The sugar-free pigment obtained above was recrystallized from a mixture of ethanol and 5 % ethanolic hydrochloric acid. All of its properties were quite similar to those of delphinidin described by Willstätter *et al.*⁵⁾. No methoxyl group was present in the molecule. R_f value was 0.23 on irrigation with acetic acid/conc. hydrochloric acid/water (5:1:5, v/v) at $28 \pm 1^{\circ}$, using Tôyô No. 52 filter paper.

Found on anhydrous specimen: C 53.29; H 3.24. Calc. for $C_{15}H_{11}O_7Cl$: C 53.18, H 3.27.

Water of crystallization. Found: H_2O 10.09. Calc. for $C_{15}H_{11}O_7Cl \cdot 2H_2O$: H_2O 9.61.

B. "Parrot Pierson"-perianths

Isolation of anthocyanin (keracyanin) as chloride. Fresh perianths (7.1 kg.) were immersed in 1 % methanolic hydrochloric acid (5 l.) for 2 days. The dark red extract was decanted and the residue was extracted again with methanol (1.5 l.) for 3 hrs. A saturated methanolic solution of basic lead acetate was slowly added to the combined filtrate (6 l.), and the bluish green precipitate formed was collected and

washed thoroughly with water and methanol. This was immediately converted into chloride by dissolving in 5 % methanolic hydrochloric acid, and the anthocyanin was precipitated with ether (10 vol.). The amorphous red precipitate was dissolved in 1 % methanolic hydrochloric acid and precipitated with ether (5 vol.). The same process was repeated further four times. Finally, the product was dissolved in 1 % ethanolic hydrochloric acid and, after addition of 5 % ethanolic hydrochloric acid (1/10 vol.), the mixture was allowed to stand for 2-5 days, whereby the anthocyanin commenced to crystallize. Recrystallization was effected by dissolution in 1 % ethanolic hydrochloric acid or warm water and addition of 5 % ethanolic hydrochloric acid.

The yield of the purest specimen was 0.45 g., corresponding to 0.006 % of fresh weight of the perianths.

Properties of "Parrot Pierson"-anthocyanin. The anthocyanin recrystallized thrice consisted of red-brown needles, and decomposed at 177° . Distribution number 6.9. Rf value found on irrigation with acetic acid/conc. hydrochloric acid/water (3:1:3, v/v) at $28 \pm 1^{\circ}$ using Tôyô No. 52 filter paper was 0.47. The solubility, absorption spectrum (cf. Fig. 2) and colour reactions showed a good agreement with those of keracyanin previously isolated from the tulip-flower ("Eclipse")⁶. Anal. Found: C 51.11, H 4.83. Calc. for $C_{27}H_{31}O_{15}Cl$: C 51.33; H 4.92. Water of crystallization. Found: H_2O 7.56. Calc. for $C_{27}H_{31}O_{15}Cl \cdot 3H_2O$: H_2O 7.98.

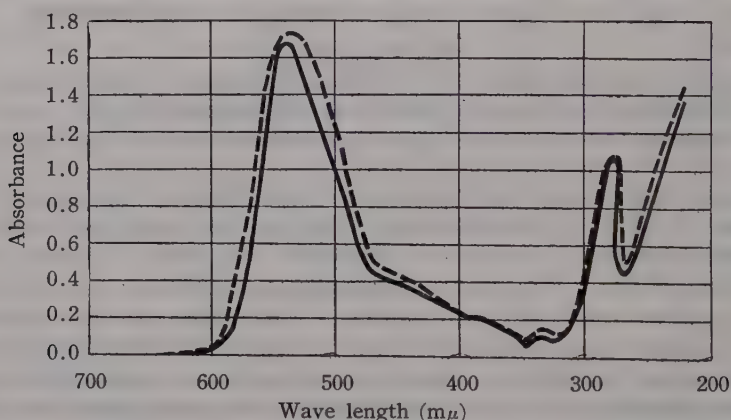


Fig. 2. Absorption spectra of "Parrot Pierson"-anthocyanin.

— "Parrot Pierson"-anthocyanin, 5/10,000 mol in 60 % EtOH (HCl-conc. 0.1 %)
 - - - - Keracyanin (from "Eclipse"), 5/10,000 mol in 60 % EtOH (HCl-conc. 0.13 %)

Hydrolysis. Anhydrous glycoside chloride (85 mg.) was dissolved in warm water (10 ml.), and the solution was added with an equal volume of 36 % hydrochloric acid and boiled for 3 min. After standing in a refrigerator overnight, dark chocolate-brown crystals were collected. Yield 43.4 mg (51.02 %). The mother liquor was shaken with *iso*-amyl alcohol to remove any trace of aglycone, and was used for the determination of sugars. The qualitative examinations by means of colour reaction, osazone formation and Rf value have demonstrated the presence of glucose and rhamose.

Aglycone (cyanidin chloride). The sugar-free pigment obtained above was recrystallized five times from ethanol containing about 5 % hydrochloric acid. It was obtained in characteristic, long red-brown needles.

On co-chromatography with cyanidin chloride obtained from *Pennisetum*⁶ and *Tulipa*², only one spot was obtained on the chromatogram, giving Rf value 0.34 by

means of acetic acid/conc. hydrochloric acid/water (5:1:5, v/v) using Tôyô No. 52 filter paper. The solubility and colour reactions also proved the identity with cyanidin.

Micro-Zeisel estimation proved the absence of methoxyl group. Anal. Found: C 55.66; H 3.41. Calc. for $C_{15}H_{11}O_6Cl$: C 55.82; H 3.43. Water of crystallization. Found: H_2O 7.29. Calc. for $C_{15}H_{11}O_6Cl \cdot 1\frac{1}{2}H_2O$: H_2O 7.72.

Finally, it may be noted that according to the paper chromatographic and spectrophotometric studies, "The Bishop"-flower contained four anthocyanins approximately in the following proportion: delphin (1 part), tulipanin (7 parts), keracyanin (2 parts) and pelargonidin glucorhamnoside (trace), and "Parrot Pierson"-flower three anthocyanins, *i.e.*, keracyanin (4 parts), pelargonidin glucorhamnoside (4.5 parts) and tulipanin (1.5 parts) (M. Shibata and N. Ishikura, unpublished results).

Summary

Two anthocyanins in the flower of garden varieties of tulip were isolated in crystalline state and their properties and structure have been described. The one from the perianth of the garden variety, "The Bishop" (strain of Darwin, bright violet, large flower) was tulipanin (delphinidin-3-glucorhamnoside), which was previously isolated by one of us (M.S.) from the dark purple perianth of a variety of tulip ("Queen of Night"), and the other from the perianth of the garden variety, "Parrot Pierson" (strain of Parrot, dark red flower), was nothing but keracyanin (cyanidin-3-glucorhamnoside), which had been isolated by us from the blood-red perianth of a variety of tulip ("Eclipse").

The authors are indebted to Prof. K. Hayashi (Tokyo University of Education) for elementary analysis, and also to Mr. I. Demura in Tonami city and Mr. N. Kobayashi in Nyuzen town for their donation of flowers used in this experiment.

References

- 1) Shibata, M., Bot. Mag. Tokyo **69**: 462 (1956).
- 2) —, and Sakai, E., *ibid.* **71**: 6 (1958).
- 3) —, and Ishikura, N., Naturwissenschaften **46**: 602 (1959).
- 4) —, and —, Jap. Jour. Bot. **17**: 230 (1960).
- 5) Willstätter, R. and Mieg, W., Ann. Chem. **408**: 61 (1914).
- 6) Shibata, M., and Sakai, E., Bot. Mag. Tokyo **71**: 193 (1958).

摘 要

柴田万年・堺恵美: チューリップの 2 品種のアントシアニンについて

さきにチューリップの花のアントシアニンを結晶状に単離し、その性質、構造などを報告したが、本報では更に他の品種——ザ・ビショップ(ダーウィン系、紫色大花)とパロット・ピアソン(パロット系、濃赤色花)——からそれぞれ色素を結晶状に単離することができた。前者からはチュリパニン(デルフィニジンのラムノグルコシド)、後者からはケラシアニン(シアニジンのラムノグルコシド)が得られた。なお、ペーパークロマトグラフィーおよび分光光度法的測定によれば前者の花には 4 種類、後者の花には 3 種類のアントシアニンが含まれていることがわかった。(富山大学文理学部生物学教室)

The Effects of 2,4-Dichlorophenoxyacetic Acid on Growth and Respiration in Yeasts*

by Kazuyoshi NISHIGAMI**

Received October 29, 1960

The 2,4-dichlorophenoxyacetic acid has been widely used as an effective herbicide. Its effects on higher plants were studied by Fang and Butts¹⁾, Akers and Fang²⁾, Morre and Rogers³⁾ and Key *et al.*⁴⁾. Concerning the effect of 2,4-D upon the respiratory activity of higher plants, Brown⁵⁾ reported that 2,4-D stimulated the respiration of seedlings of bean plants. Kelly⁶⁾ found that the respiration of pea stem tissues was accelerated by low concentrations of this substance. Further, Humphreys and Dugger^{7,8)} studied the effect of 2,4-D on respiration in pea, corn and oat seedling.

On the other hand, in lower plants such as algae and bacteria, the effect of 2,4-D on their respiratory activity has also been reported; it was proved that the respiration of *Spirogyra*⁹⁾, *Chlorella*¹⁰⁾, *Azotobacter* and *Rhizobium*^{11,12)} was stimulated by low concentrations of 2,4-D.

As far as the author is aware, yeasts have never been used in the study of 2,4-D. The present study deals with the effect of 2,4-D on the growth and respiratory activity of yeasts.

Materials and Methods

Three kinds of yeasts were used: *Rhodotorula glutinis* (wild yeast), *Saccharomyces cerevisiae* (baker's yeast) and *Saccharomyces carlsbergensis* (bottom brewer's yeast).

The yeasts were inoculated on 50 ml. of Henneberg solution contained in 300 ml. Erlenmeyer flasks and cultured at 30°. The details of culture methods used were identical with those described in a previous paper¹³⁾. In the present studies both the shaking culture (aerobic condition) and the static culture (relatively anaerobic condition) were used. Yeasts were grown in a solution containing 2,4-D (final concentration between 0.004 M and 0.046 M), and were collected by centrifugation at 4,000 r.p.m. at the end of the growth period. Then they were washed three times with distilled water, dried in an air bath, and their dry weights were measured.

In the test of respiration of yeasts, the cells were collected at logarithmic phase and the usual Warburg procedures were employed. The materials and their final molar concentration in each reaction chamber were as follows: cells suspension, 0.5 ml.; glucose as substrate, 0.025 M; phosphate buffer, pH 5.4, 0.025 M; 2,4-D as sodium salt, from 0.01 M to 0.05 M. The volume of solution in the reaction chamber was adjusted with distilled water to make 2 ml. in total. The center well contained 0.5 ml. of 20 per cent KOH and a fluted filter paper strip. The gas phase was air and the temperature was 30°.

Results

Effects of 2,4-D on the growth of yeasts.

The inhibitory effects of 2,4-D on the growth of three kinds of yeasts are shown

* Dedicated to Prof. Hajime Matsuura and Prof. Yukio Yamada celebrating their sixtieth birthdays.

** Biological Institute, Faculty of Literature and Science, Shimane University, Matsue, Japan.

in Figs. 1, 2 and 3. As will be seen in Fig. 1, the wild yeast, *Rhodotorula glutinis*, is more sensitive to 2,4-D than other yeasts, *Saccharomyces cerevisiae* and *Saccharomyces carlsbergensis*. The growth of *R. glutinis* under the shaking culture was generally greater than that under static condition whether in the presence of 2,4-D or in its absence. In Fig. 2, it is indicated that baker's yeast is more sensitive to 2,4-D, if the yeast is cultivated under the shaking condition. Fig. 3 shows that brewer's yeast, *S. carlsbergensis*, is the most resistant species to 2,4-D among the three kinds of yeasts.

Effects of 2,4-D on the respiration of yeasts.

Effects of 2,4-D on the respiration of the three kinds of yeasts are shown in Figs. 4, 5 and 6. The respiration of *R. glutinis* was stimulated by low concentrations of 2,4-D. On the contrary, high concentrations of 2,4-D inhibited the respiration. On *S. cerevisiae* the respiration of exogenous glucose was stimulated fairly by 2×10^{-2} and 3×10^{-2} M of 2,4-D, but only a little effect was observed at higher

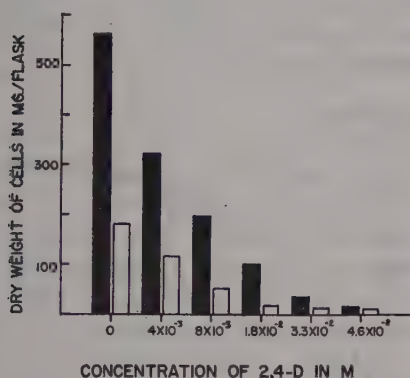


Fig. 1. The effects of various concentrations of 2,4-D on the growth of *Rhodotorula glutinis*. Solid bar: shaking culture. Clear bar: static culture.

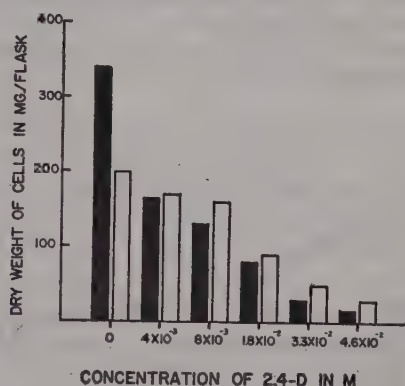


Fig. 2. The effects of various concentrations of 2,4-D on the growth of *Saccharomyces cerevisiae*. Solid bar: shaking culture. Clear bar: static culture.

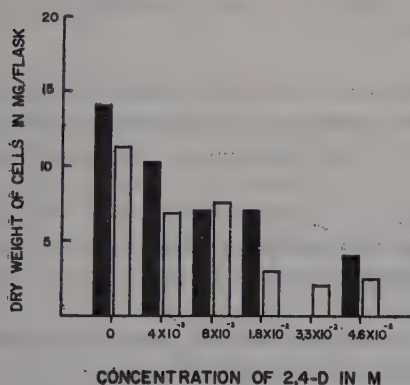


Fig. 3. The effects of various concentrations of 2,4-D on the growth of *Saccharomyces carlsbergensis*. Solid bar: shaking culture. Clear bar: static culture.

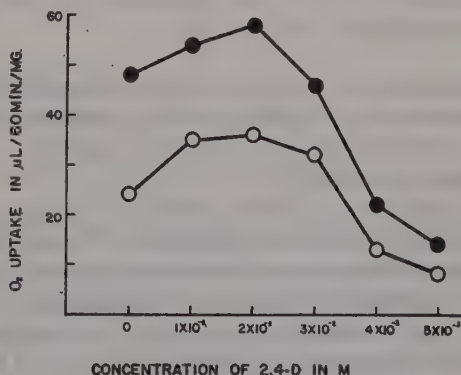


Fig. 4. The effects of various concentrations of 2,4-D on the respiration of resting *Rhodotorula glutinis* cells. Closed circles: respiration of glucose. Open circles: endogenous respiration.

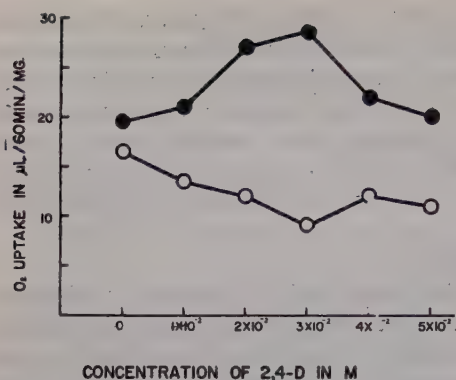


Fig. 5. The effects of various concentrations of 2,4-D on the respiration of resting *Saccharomyces cerevisiae* cells. Closed circles: respiration of glucose. Open circles: endogenous respiration.

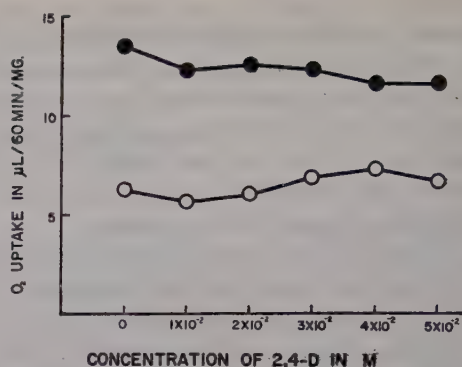


Fig. 6. The effects of various concentrations of 2,4-D on the respiration of resting *Saccharomyces carlsbergensis* cells. Closed circles: respiration of glucose. Open circles: endogenous respiration.

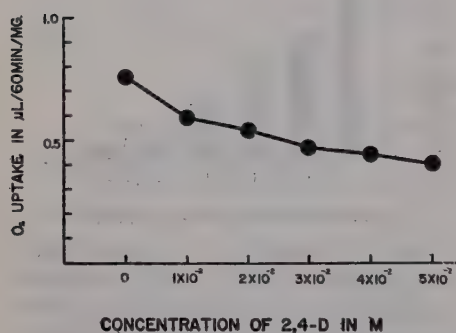


Fig. 7. The effects of various concentrations of 2,4-D on the respiration of dried *Saccharomyces cerevisiae* cells.

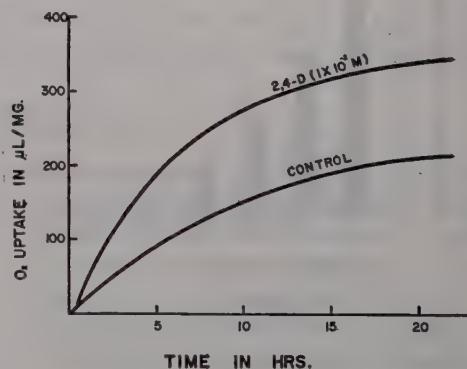


Fig. 8. The stimulatory effect of 2,4-D on the respiration of resting *Rhodotorula glutinis* cells.

concentrations. However, the endogenous respiration was not stimulated in any concentration tested but it was inhibited. The respiration of exogenous glucose on *S. carlsbergensis* was inhibited slightly by 2,4-D. Its endogenous respiration was almost unaffected.

Effect of 2,4-D on respiration of dried yeast.

Fig. 7 indicates the effect of 2,4-D on the respiration of dried baker's yeast. The respiration was fairly inhibited by a low concentration of 2,4-D.

Durability of stimulated respiration.

Fig. 8 shows the durability of stimulated respiration of wild yeast, *R. glutinis*, which demonstrates that the effect of 2,4-D continued for about 10 hours.

Discussion

The effect of 2,4-D on the growth of yeast used in these investigations was inhibitory rather than stimulatory at any concentrations tested (from 4×10^{-3} to 4.6

$\times 10^{-2}$ M). Especially, the growth of *R. glutinis* was inhibited greatly as compared with the other two kinds, *S. cerevisiae* and *S. carlsbergensis*.

According to Johnson *et al.*^{11,12}, the respiration of *Azotobacter vinelandii* was inhibited perfectly with 9.2×10^{-3} M of 2,4-D. Wedding *et al.*¹⁰ reported that the respiration of *Chlorella* was inhibited by 5×10^{-4} M or higher concentrations of 2,4-D. Suematsu⁹ also observed that the respiration of *Chlorella* was inhibited perfectly by 1 per cent (about 2×10^{-2} M) of 2,4-D.

In the present study, it was indicated that the respiration of yeasts was more resistant to 2,4-D than are *Azotobacter* and *Chlorella*. On *R. glutinis* the respiratory activity was stimulated even by 1×10^{-2} , 2×10^{-2} and 3×10^{-2} M of 2,4-D, and never inhibited. The inhibition occurred only at 4×10^{-2} M. Moreover, the respiration of *S. cerevisiae* and *S. carlsbergensis* was more resistant to 2,4-D than that of *R. glutinis*. The resistance of yeast against 2,4-D may be attributed to the non-permeability of cell membrane, however, further investigation is necessary for elucidation of this problem. There appears a relationship between respiratory activity and respiratory inhibition by 2,4-D in these yeasts. Those with stronger respiration seem more susceptible to the inhibitory action of 2,4-D in higher concentrations than the yeasts with lower respiratory activities.

In relatively low concentration (4×10^{-3} M) of 2,4-D the growth of all yeasts is inhibited. This effective concentration is higher than the concentration (9.2×10^{-3} , 5×10^{-4} M) effective in *Azotobacter* as reported by Johnson^{11,12} and Wedding¹⁰, but is lower than the concentration (from 1×10^{-2} to 5×10^{-2} M) effective in respiratory inhibition. 2,4-D in 1×10^{-3} M inhibited the growth of *R. glutinis*, on the other hand, 2×10^{-3} M of 2,4-D stimulated its respiratory activity.

With regard to growth and respiration of yeasts, it was found that *R. glutinis* and *S. cerevisiae* were more sensitive to 2,4-D than *S. carlsbergensis*. And it was concluded that the growth of yeasts is more sensitive to 2,4-D than respiration is.

An unexpected result was obtained on the effect of 2,4-D upon respiration of dried baker's yeast, *S. cerevisiae*, in the present study. In the case of intact yeast the respiration of this yeast was stimulated by 2×10^{-2} to 3×10^{-2} M of 2,4-D. Nevertheless, there was no stimulatory effect of the dried cells in these concentrations. It is considered that intact cell-structure may be necessary for the stimulation of respiration.

Summary

The effects of 2,4-D on growth and respiration of three kinds of yeasts were studied.

The growth of *R. glutinis* and *S. cerevisiae* was more sensitive against 2,4-D than that of *S. carlsbergensis*.

A stimulatory effect on the respiration of *R. glutinis* and *S. cerevisiae* was observed in relatively high concentrations. On the contrary, the endogenous respiration of *S. cerevisiae* was not stimulated but inhibited. The respiration of *S. carlsbergensis* was slightly affected.

On dried baker's yeast, the respiration was not stimulated at all but rather it was inhibited.

The respiration of intact cells of *R. glutinis* was stimulated for about 10 hours.

The author is greatly indebted to Professor S. Usami of Hokkaido University for his never-failing interest and support throughout the work. The author also

wishes to express his thanks to Dr. T. Hasegawa of Institute for Fermentation, Osaka, for his kind advice in the identification of species.

References

- 1) Fang, S. C., and Butts, J. S., *Plant Physiol.* **29**: 365 (1954). 2) Akers, T. J., and Fang, S. C., *ibid.* **31**: 54 (1956). 3) Morre, D. J., and Rogers, B. J., *ibid.* **35**: 324 (1960). 4) Key, J. L., Hanson, J. B., and Bils, R. F., *ibid.* **35**: 177 (1960). 5) Brown, J. W., *Bot. Gaz.* **107**: 332 (1946).
- 6) Kelly, S., *Amer. Jour. Bot.* **36**: 421 (1949). 7) Humphreys, T. E., and Dugger, W. M., *Plant Physiol.* **32**: 136 (1957). 8) —, *ibid.* **32**: 530 (1957). 9) Suematsu, I., *Jour. Hokkaido Gakugei Univ. B*, **6**: 21 (1955). 10) Wedding, R. T., Erickson, L. C., and Brannaman, B. L., *Plant Physiol.* **29**: 64 (1954). 11) Johnson, E. J., and Colmer, A. R., *Jour. Bact.* **73**: 139 (1957).
- 12) —, *ibid.* **73**: 666 (1957). 14) Nishigami, K., *Jour. Shimane Univ.* **10**: 110 (1960).

摘 要

西上一義： 酵母の生長と呼吸とにおよぼす 2,4-D の影響

3 種類の酵母, *Rhodotorula glutinis*, *Saccharomyces cerevisiae* および *Saccharomyces carlsbergensis* の生長と呼吸におよぼす 2,4-D の影響をしらべた。

4×10^{-3} M ないし 4.6×10^{-2} M の 2,4-D を与えた生長実験では酵母はつねに阻害を受けた。なかでも呼吸能の高い酵母ほど阻害率が高かった。

呼吸に対する影響では、呼吸能の比較的に高い酵母 *R. glutinis* は比較的低濃度の 2,4-D で呼吸促進の効果がみられ、高濃度では阻害を受けた。呼吸能の比較的に低い酵母 *S. carlsbergensis* ではあまり影響がみられなかった。

乾燥酵母では呼吸促進効果がみられなかった。

R. glutinis に対する呼吸促進効果は、約 10 時間続いた。

一般に酵母に対して影響を与えるためには、*Azotobacter* や *Chlorella* と比べて高濃度の 2,4-D を必要とする。(島根大学文理学部生物学教室)

ツルモの遊走子形成*

西林長朗**・猪野俊平**

Takeo NISHIBAYASHI** and Shumpei INOH**: The Formation of Zoospores in *Chorda filum* (L.) Lamour.*

1960 年 10 月 12 日受付

著者らはコンブ目植物の遊走子嚢発生および遊走子形成過程の観察を計画し、すでにワカメ¹⁾、ミツイシコンブ²⁾、スジメ³⁾、ヒロメ⁴⁾についての遊走子形成の観察結果を報告した。今回は北海道から九州にかけて、わが国のいたるところに繁茂しているツルモ (*Chorda filum*) を材料に用いて、その遊走子形成の細胞学的な観察を行なった。ツルモはコンブ目の中でも、その体構造が最も簡単であり、分類学上、最下位におかれている植物である。

Kylin (1918)⁵⁾はスウェーデン産の *Chorda filum* を用いて、組織学的ならびに発生学的な研究を綿密に行なっており、遊走子形成の際の核分裂および細胞分裂にも論及している。わが国では、神田 (1938)⁶⁾がツルモの遊走子を培養して、配偶体の発達過程をくわしく観察している。しかし、まだ遊走子形成の際の細胞学的な研究は、わが国では報告されていない。また今回の著者らの観察の結果から、Kylin の報告とは異なる新しい知見が得られたので、それをここに報告する。

材料と方法

本研究に用いた材料は、瀬戸内海の塩飽群島に生息しているツルモ (*Chorda filum*) であり、1956年

4月23日、1957年4月27日および1959年4月22日の3回にわたって採集を行なった。ツルモは1本の紐のような細長い、枝分れのない葉状体であり、その遊走子嚢はコンブやワカメのように葉状体のある部位に集中した子嚢斑をつくらないで、葉状体の体表全面につくられる。遊走子嚢は葉状体の基部から、徐々に上方に向かうにしたがって分化していく。このような孢子嚢群の若いところを選び出し、その部分を小さく切って阿部氏液で固定した。固定時間は20~35時間である。その後、普通のパラフィン切片法により4~5 μ の切片をつくり、10%過酸化水素水で約35時間漂白した後、ハイデンハイン氏鉄みょうばんヘマトキシリンで染色して観察を行なった。

観察および考察

Fig. 1 は下位細胞から切り出されて間もない若い遊走子嚢細胞 (sporangial cell) であり、その中には静止核と2~3個の色素体が含まれている。核が分裂をはじめると、核はその容積を増すとともに、核内には染色糸の網目が次第に明瞭になってくる (Fig. 2)。核内には多くの場合、1個の球形の仁が観察されるが、約2.6%の割合で二つの仁を有するものが認められた (Fig. 3)。染色糸は染色性を増してくるとともに、核内腔の一隅に集まってシナプシス期にはいる (Fig. 4)。染色糸のいくらかのものはつねに仁に付着している。その後、染色糸は核腔内全体にひろがり、そこに核糸の網目を形成する (オープン・スピレム期) (Fig. 5)。核腔内にひ

* 文部省科学研究費、課題番号 407130、岡山大学理学部生物学教室植物形態学研究業績 No. 79、玉野臨海実験所業績 No. 75。

** 岡山大学理学部生物学教室 Department of Biology, Faculty of Science, Okayama University, Okayama, Japan.

ろがった染色体は各所で短縮、肥厚して O, Y, V, II 状の二価染色体が観察されるようになる (Figs. 6, 7). この時期がディアキネシス期であり、核の大きさは分裂の各期を通じてこの頃が最大となり、その直径は約 6μ となる。仁はこの頃までには完全に消失する。その後もなお染色体の短縮、肥厚は続き、染色体は小さな粒状となって核腔内に散在するようになる。この時、約 30 の二価染色体を数えることができた (Figs. 8, 9).

中期では、核膜は不明瞭になるとともに、各二価染色体は赤道板に整然とならぶようになる。側面観では、これらの染色体はその中央でくびれてあれい状を呈している。また紡錘体が明瞭に観察される (Fig. 11). Kylin (1918)⁵⁾ はツルモの生長帯の体細胞分裂において、紡錘体の極に各一つずつの中心体の存在を報じている。しかし、著者らは注意深く観察を行なったけれども、中心体を認めることはできなかった。中期の極面観像では、核板はいちぢるしく収縮して染色体はたがいにくっつき合っている (Fig. 12). 染色体が赤道板にならび終わった後まで、核膜が消失しないで残っている場合が観察された (Fig. 10). 紡錘体の軸の方向は、遊走子囊の長軸に平行なこともあれば直角のこともあって、分裂軸の方向は一定していない。

赤道板にならんでいた各二価染色体は、一価染色体に分けられて規則正しく両極に向かって移動する (Fig. 13). 染色体が紡錘体の極付近に達した頃には、染色体は集まってかたまりとなり、個々の染色体は識別できなくなる (Fig. 14). 二つの染色体のかたまりの間には、なお紡錘糸が観察される。

終期には、核膜も仁も再び現われて、核は静止期で見られたのと同じような状態となる。核膜が再成された時には、2 娘核は接近しているが、後には移動して離れていく (Fig. 15).

2 娘核の間には隔膜が形成されることなく、ただちに第二分裂がはじまり、遊走子囊細胞内には遊離した 4 核が形成される (Fig. 17). 第二分裂における二つの核の分裂は同時的であるが、分裂の方向はおたがいに関係がなく、二つの分裂軸が平行なこともあれば、たがいに直角の位置を占める場合もある (Fig. 16).

第二分裂にひきつづいて、第三、第四分裂が行なわれて、遊走子囊細胞内には 16 の遊離核が形成さ

れる (Figs. 19, 21). これらの分裂においても、細胞内の核はすべて同時的に分裂する (Figs. 18, 20). 核分裂のくりかえしによって、核の数が増すとともに、色素体の数も増加して、第四分裂終了の時には色素体の数も 16 となる。胞子囊内に散在している 16 の核のおのおのに、一つずつの色素体が移動していく。その後、細胞質が核のまわりに集まり、細胞質の外側は薄い膜で境されて、胞子囊内には 16 の遊走子が形成される (Fig. 22). 第四分裂の中期頃から、胞子囊の頂端の膜は肥厚しはじめ、コンブ目植物の胞子囊の特徴である粘液帽の形成が見られる。しかし、この膜の肥厚の程度は僅少であって、コンブ、ミツイシコンブ、スジメのように著しくはない。完熟した遊走子囊は長さ $28\sim 38\mu$ 、幅 $8\sim 12\mu$ であり、その中には一つの色素体と 1 核をもった 16 の遊走子がいっている。

以上の観察の結果、第一分裂前期にシナプシス、オープン・スピレム、ディアキネシスの各期が確認されたので、ツルモの遊走子囊内の第一および第二分裂が減数分裂である。Kylin⁵⁾ はツルモの生長帯における体細胞分裂で 40 の染色体を数え、遊走子囊内の第一核分裂で約 20 の染色体を認めている。著者らの今回の観察の結果では、本邦産のツルモの染色体数は半数で約 30 と決定されるので、この染色体数の相違は研究に用いた材料の生育場所の違いによるのか、あるいは固定の操作を含めたプレパラート作製上の方法の違いによるのか、さらに今後の研究によって決定したい。

一つの遊走子囊内に作られる遊走子の数は Kylin の報告と同様に 16 である。コンブ目植物の多くのものでは、32 の遊走子が作られる。ツルモでは遊走子形成のさいの核分裂が 1 回省略されて、16 の遊走子を生じるということ、またツルモの胞子囊群が葉状体の全面にわたってつくられることは興味あることと思われる。神田 (1938)⁶⁾ はツルモの遊走子は眼点をもち、配偶子のべん毛は不等長であり、若い胞子体の頂端部および側方から多数の毛を生じ、またその仮根は隔膜によって、いくつかの部分に仕切られているなど、ツルモを除いた他のコンブ目植物では見ることでできない多くの特徴を報じている。しかし、この植物の半数染色体数は約 30 で、スジメ、ワカメ、ヒロメと同じである。



Plate I. Formation of zoospores in zoosporangia of *Chorda filum* (L.) Lamour. All magnifications ca. $\times 2600$.

Fig. 1. Resting stage. Fig. 2. Later stage with reticular structure. Fig. 3. The same stage, showing two nucleoli in the nuclear cavity. Fig. 4. Synapsis stage. Fig. 5. Open spireme stage. Fig. 6. Early diakinesis. Fig. 7. Diakinesis, showing various shaped bivalent chromosomes. Figs. 8, 9. Late diakinesis. Figs. 10, 11. Side view of the metaphase. In Fig. 10, nuclear membrane is still seen. Fig. 12. Polar view of the metaphase.

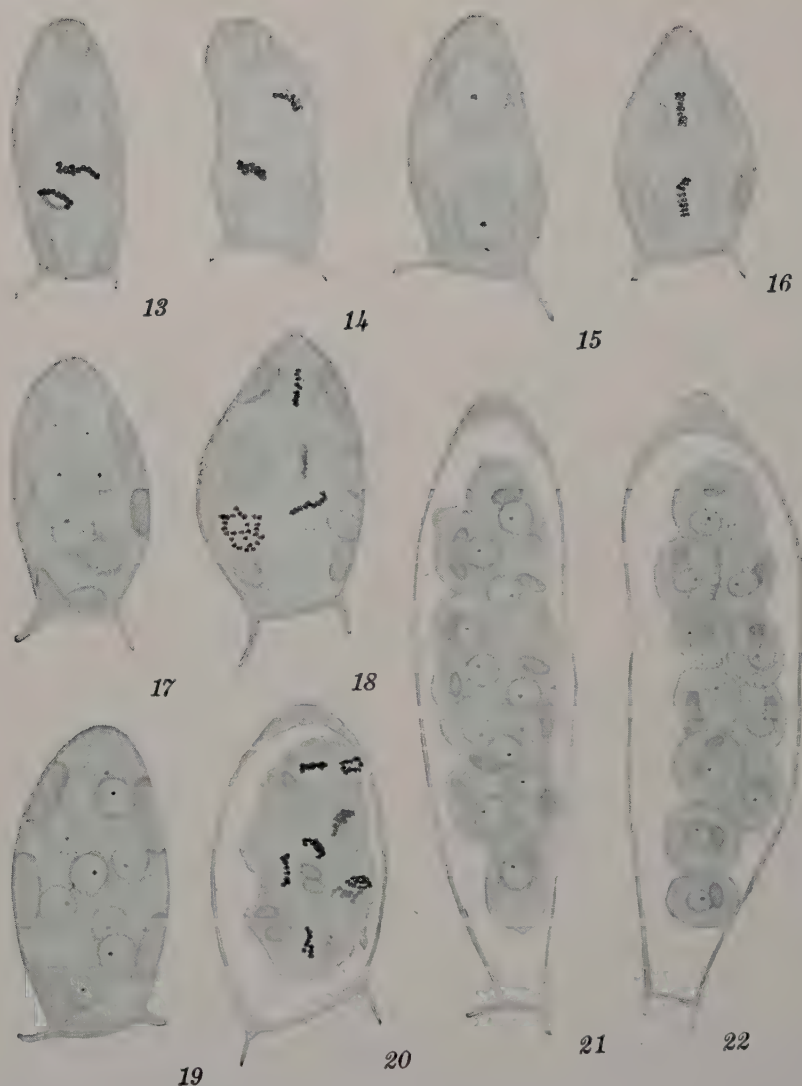


Plate II. Formation of zoospores in zoosporangia of *Chorda filum* (L.) Lamour. All magnifications ca. $\times 2600$.

Figs. 13, 14. Anaphase. Fig. 15. Two daughter nuclei. Fig. 16. Metaphase of the second meiotic division. Fig. 17. Four-nucleate stage. Fig. 18. Metaphase of the third nuclear division. Fig. 19. Eight-nucleate stage. Fig. 20. Metaphase of the fourth nuclear division. Fig. 21. Sixteen-nucleate stage. Two nuclei are latent. Fig. 22. Zoospores in a zoosporangium.

摘 要

1. ツルモ (*Chorda filum*) の遊走子嚢細胞内の第一核分裂で、シナプシス、オープン・スピレム、ディアキネシスの各期が観察され、第一および第二分裂が減数分裂である。

2. 本種の半数染色体数は約 30 である。

3. 紡錘体は明瞭に観察されるが、中心体は認められない。

4. 減数分裂の後、ひきつづいて2回の核分裂がおこなわれて、遊走子嚢内には 16 の遊離核が形成される。その後、おのおのの核を中心として遊走子がつくられるので、遊走子嚢内には 16 個の半数性の遊走子が含まれている。

文 献

- 1) 猪野俊平・西林長朗, 染色体 **22-24**: 788 (1955).
- 2) 西林長朗・猪野俊平, 植雑 **69**: 501 (1956).
- 3) ————, 同 **70**: 228 (1957).
- 4) ————, 同 **73**: 494 (1960).
- 5) Kylin, H., Svensk. Bot. Tidskr. **12**: 1 (1918).
- 6) Kanda, T., Sci. Pap. Inst. Algol. Res., Fac. Sci. Hokkaido Imp. Univ. **2**: 87 (1938).

Summary

1. In the first nuclear division in the zoosporangial cell of *Chorda filum*, synapsis, open spireme, and diakinesis stages are observed. The first and second nuclear divisions are meioses.

2. The haploid chromosome number of this alga is about thirty.

3. The centrosome is not observed, but the spindle is visible.

4. After meiosis, two successive mitoses take place to produce 16 free nuclei. Each zoosporangium contains 16 haploid zoospores.

Short Communication

Hideo TOYOKUNI*: Séparation de *Comastoma*, genre nouveau, d'avec *Gentianella*

豊国秀夫*: 新属サンブクリンドウ属

Reçu le 23 février, 1961

La section *Comastoma* du genre *Gentiana*, créée par Wettstein en 1896, fut transférée au genre *Gentianella* par H. Smith, comme il examina le genre *Gentiana*, s.l. en Chine, et suivit l'opinion proposée par Schustler (1923) en 1936. En 1956, Á. et D. Löve transférèrent cette section de *Gentianella* en *Lomatogonium*, tandis que Gillett la regarda comme un sous-genre du genre *Gentianella* en 1957.

Cependant, à mon avis, ce groupe n'appartient ni à *Gentianella* ni à *Lomatogonium*, il représente plutôt un genre à part.

Voici une diagnose de ce genre nouveau avec quelques transferts techniques accompagnant la publication de ce genre. Des détails ultérieurs seront débattus dans mon travail monographique des Gentianacées japonaises.

Comastoma Toyokuni, genus novum.

Syn. *Gentiana* sect. *Endotricha* Froel., De Gent. 86. 1796. pro parte. *Gentiana* sect. *Amarella* Griseb., Gen. & Sp. Gent. 238. 1839. pro parte. *Gentiana* sect. *Comastoma* Wettst. in Österr. Bot. Zeits. 46: 174. 1896. *Lomatogonium* sect. *Comastoma* (Wettst.) Á. & D. Löve in Acta Hort. Gotob. 20: 117. 1956. *Gentianella* subg. *Comastoma* (Wettst.) Gillett in Ann. Miss. Bot. Gard. 44: 262. 1957.

Cum generibus *Gentianellæ Lomatogonii*-que hoc genus novum comparandum est; tamen, corollæ fimbriatis squamis, non vasalibus fibris percurrentibus, fimbrillis squamarum linearibus nec filiformibus, et tribus non quinque fibris vasalibus in quoque corollæ lobo, et duobus nectariis epipetalis, dum *Gentianella* unum habet. Corollæ loborum squamis cum nectariis epipetalis non junctis, squamis et nectariis epipetalis longe distantioribus, fibris vasalibus in quoque corollæ lobo paucis, solum tribus, quo numero genus novum a *Lomatogonio* distat.

Flores tetrameri v. quinarii, plerumque longipedunculati, calycibus profunde 4-5-partitis, tubis brevissimis, corollis campanulatis v. hypocraterimorphis, apicibus 4-5-lobatis, ad basin loborum ornatis squamis fimbriatis et bifissis nec fibris vasalibus percurrentibus, fimbrillis squamellarum linearibus et vulgo obtusis, filamentis plerumque minute pilosellis, stylo nullo, stigmatibus duobus brevissimis.

Type: *Comastoma tenellum* (Rottb.) Toyokuni

1. *C. falcatum* (Turcz.) Toyokuni, c.n.—*Gentiana falcata* Turcz., Cat. Baik. n. 783. 1837. nom. nud.; in Bull. Soc. Nat. Mosc. 15: 404. 1842.

2. *C. limprichtii* (Grün.) c.n.—*Gentiana Limprichtii* Grün. in Fedde, Repert. 12: 308. 1913.

3. *C. nanum* (Wulf.) c.n.—*Gentiana nana* Wulf. in Jacq., Misc. 1: 161, t. 18, f. 3. 1778.

4. *C. pulmonarium* (Turcz.) c.n.—*Gentiana Pulmonaria* Turcz. in Flora 1834, Beibl. 1: 19. nom. nud.; in Bull. Soc. Nat. Mosc. 22: 317. 1849. Subsp. *arrectum* (Fr.) c. et st. n.—*Gentiana arrecta* Fr. in Journ. Linn. Soc. Bot. 26: 124. 1890. Les fimbrillæ de la couronne fimbriée de cette sous-espèce sont beaucoup plus nombreuses que celles de l'espèce typique. Subsp. *sectum* (Satake) c.n.—*Gentiana Takedai* var. *secta* Satake in Journ. Jap. Bot. 16: 423, f. 2. 1940.

5. *C. tenellum* (Rottb.) c.n.—*Gentiana tenella* Rottb. in Kjöb. Selsk. Skrift. 10: 436, t. 2, f. 6. 1770.

* Institut Botanique, Faculté des Sciences, Université d'Hokkaidô, Sapporo, Japon.

Ecological Studies of Sasa Communities

I. Productive Structure of Some of the Sasa Communities in Japan*

by Yasuyuki OSHIMA**

Received October 21, 1960

Bamboo grasses, "Sasa" in Japanese, include the species belonging to the genera, *Sasa*, *Sasamorph*a, *Pleioblastus*, *Arundinaria*, etc. Most of these genera are distributed endemically in the Far East, e.g., *Sasa* is an endemic genus in Japan, Korea and Sakharin, *Sasamorph*a in Japan and Manchuria, and *Pleioblastus* in Japan, and many species are restricted to Japan where they grow almost everywhere. In the montane and subalpine zones they are often the dominant species of grassland, and more often of the undergrowth of deciduous broad-leaved forests and sometimes of subalpine coniferous forests. According to the 1950 year report of the Forestry Agency of Japan, Sasa covers areas of about 50% of the national forest land of Japan, particularly about 90% in Hokkaido.

They often invade into the open forest, especially in the site where the forest has newly been destroyed by cutting, fire or other agencies. On the other hand, they constitute frequently almost or utterly pure communities on the lower slopes of volcanoes, and maintain their dominancy for a long time by virtue of their low requirement to the environment and their superiority in the competition with other grasses and herbs, or sometimes with tree species.

Up to the present many studies have been made concerning the geographical distribution of Sasa species and the destruction of Sasa communities from the necessity of forestry, but only a few ecological and phytosociological papers have been published. Yoshioka¹⁾ studied mainly synecological characters of Sasa communities, clarifying the relationships between standing crops or culm numbers and habitat factors at Mt. Hakkoda in the northern Honshu. From the viewpoint of forestry Ueda and Uchimura²⁾ studied recently relationships between culm elongation and climatic factors, seasonal change of reserved starches correlated with culm and rhizome growth, and the effective stage for weeding of *Pleioblastus pubescens* from the forest ground.

These studies have provided us much information and suggestions on the relation between the growth of Sasa and the environmental factors. However, they were rather descriptive and the information on the mechanism of the stability of their dominancy was very little. In order to elucidate this problem, the author has made an attempt to analyze the growth of Sasa and Sasa community with different dominant species under field conditions on the basis of dry matter production which was introduced by Boysen Jensen³⁾ and on which many precise studies⁴⁻¹⁹⁾ have been carried out recently in respect to the growth of plant communities.

* A part of expense of this study was supported by a Grant-in-Aid from the Research Fund of the Ministry of Education.

** Department of Biology, Faculty of Science, Tokyo Metropolitan University, Setagaya, Tokyo, Japan.

In the present paper, closed *Sasa* communities of various localities will be discussed about their standing crop, productive structure, leaf area index, and the light condition in the communities in summer when a maximum matter production is expected, together with the description of environmental factors of the stations.

Description on the Environmental Factors of the Stations

1. Location and sketch of the vegetation

Station A: Mt. Kirigamine is a dead volcano with gentle slopes, situated in Nagano Prefecture. The upland meadow of vast area consisting of grasses and herbs develops over the greater part of the mountain, and communities dominated by *Sasa nipponica* are seen in many places of the grassland. The station was settled at Tomezuka about 1700 m. in altitude, the southern part of the grassland, in an almost pure community of *S. nipponica*.

Station B: Mugikusa Pass (2150 m. above the sea level) is situated between Mt. Maruyama and Mt. Chausu in the northern part of the dead volcanoes Yatsugatake in Nagano Prefecture, 16 km. SE from Mt. Kirigamine. There grows *Sasa nikkoensis* in the subalpine grassland surrounded by subalpine coniferous forests of *Abies Veitchii*, *A. Mariesii* and *Tsuga diversifolia*, and also on the forest ground forming a dense community.

Station C: Ozegahara is situated at about 1400 m. in altitude, 150 km. N from Tokyo, and surrounded by dead volcanoes of 2000–2350 m. high. It is known as one of the typical high moors in Japan. Deciduous forests of *Fagus crenata* and *Quercus crispura* cover the mountainsides surrounding the moor. *S. kurilensis* and *S. oseana* dominate in the shrub strata of the forest^{20, 21}), and moreover, the latter species invades either in the environs of the moor or on the side of streams running through the moor. The sampling station was selected in the dense community of *S. oseana* under the *Ulmus Davidiana* and *Betula platyphylla* var. *japonica*^{22, 23}) near the River Nujiri.

Station D: Mt. Waisuhorun (1046 m. above the sea level) is a mountain of the Niseko dormant volcano group, Hokkaido, the highest peak (1309 m.) of which is Mt. Nisekoannupuri situated at a distance of 60 km. W from Sapporo. The upper parts of these mountains higher than 400 m. are almost covered by pure communities of *S. kurilensis* (in wide sense) 2.5–3.5 m. high, and open birch forests are dotted in these communities. The *Sasa* communities here seem to be a stable vegetation caused by the past overcutting or fire of forests. The station was selected on the north-east slope from 500 to 800 m. in altitude.

2. Climate

Station A and B: The air temperatures at Tomezuka and Mugikusa have been calculated from the meteorological data at Mt. Kurumayama (1929 m. in altitude) using a lapse rate of $0.71^{\circ}/100\text{ m.}$ ²⁴) (Table 1). As to temperature factors the former closely resembles Kushiro in Hokkaido ($42^{\circ}59' \text{ N}$; min. temp. -7.1° in Jan., max. temp. 18.3° in Aug., mean ann. temp. 5.2°), and the latter is a little higher than Poronaisk in Sakhalin ($49^{\circ}12' \text{ N}$; min. temp. -17.5° in Jan., max. temp. 15.9° in Aug., mean ann. temp. 0.0°). Precipitations at both stations seem to resemble those at Mt. Kurumayama because of close distance among them (Table 2). The mean duration of snow season is four months from the middle of December till the middle of April, and the mean maximum snow depth in midwinter may be about 1 m., but this figure must fluctuate to some extent.

Table 1. Mean air temperatures at Station A, B, C and D, which were calculated from the meteorological data at observatories near the stations (see Fig. 1 and the text).

	Kuruma- yama (1944-46)	Station A Tomezuka	Station B Mugikusa	Yama- guchi (1951-55)	Mina- kami (1951-55)	Station C Ozegahara	Kutchan (1955-59)	Station D Waisuhorun	
Altitude	1925 m.	1700 m.	2150 m.	530 m.	580 m.	1400 m.	176 m.	500 m.	800 m.
Jan.	-10.5°	-8.9°	-12.1°	-3.1°	-0.8°	-6.4°	-6.4°	-8.2°	-9.8°
Feb.	-10.7	-9.1	-12.3	-3.0	-0.3	-6.3	-5.4	-7.2	-8.8
Mar.	-6.3	-4.7	-7.3	-1.9	3.9	-1.8	-2.3	-4.1	-5.7
Apr.	0.9	2.5	-0.7	7.9	9.7	4.1	3.9	2.1	0.5
May	5.8	7.4	4.2	14.8	15.1	10.2	10.4	8.6	7.0
Jun.	11.3	12.9	9.7	18.3	18.2	13.5	14.2	12.4	10.8
Jul.	14.4	16.0	12.8	22.3	22.2	17.5	19.3	17.5	15.9
Aug.	15.7	17.3	14.1	23.7	23.9	19.1	19.9	18.1	16.5
Sep.	11.6	13.2	10.0	18.9	19.2	14.3	15.2	13.4	11.8
Oct.	6.3	7.9	4.7	12.7	13.8	8.5	9.2	7.4	5.8
Nov.	0.5	2.1	-1.1	5.2	7.6	1.7	2.0	0.2	-1.4
Dec.	-8.6	-7.0	-10.2	0.3	3.3	-2.9	-3.0	-4.8	-6.4
Ann. mean	2.5°	5.1°	0.9°	10.0°	11.3°	5.9°	6.4°	4.6°	3.0°

Table 2. Average precipitations at the meteorological observatories near the stations.

	Kurumayama (1945-47) (for Sts. A, B)	Yamaguchi (1951-55) (for St. C)	Minakami (1951-55)	Kutchan (1955-59) (for St. D)
Jan.	23 mm.	179 mm.	147 mm.	213 mm.
Feb.	59	116	107	121
Mar.	89	86	97	119
Apr.	130	79	118	104
May	136	105	152	61
Jun.	194	157	232	70
Jul.	240	179	233	109
Aug.	167	179	196	174
Sep.	179	122	197	146
Oct.	283	120	139	156
Nov.	88	144	127	176
Dec.	99	168	96	226
Ann.	1683 mm.	1634 mm.	1841 mm.	1675 mm.

Station C: The air temperature at Ozegahara was calculated from the mean value of the data at two nursery gardens which are attached respectively to Yamaguchi (37° 13' N; 530 m. in altitude, about 40 km. NE from Ozegahara) and Minakami District Forestry Office (36° 47' N; 580 m. in altitude, about 30 km. SW from Ozegahara), using the mean lapse rate of 0.55°/100 m.²⁵) (Table 1 and 3). The temperatures here closely resemble those of Abashiri in Hokkaido (44° 01' N; min. temp. -7.3° in Feb., max. temp. 19.9° in Aug., mean ann. temp. 5.8°). Ozegahara may be almost the

same or somewhat higher in precipitation than Yamaguchi, because of the heavy snowfall in winter well known in the coastal region of the Japan Sea of Honshu. The maximum snow depth is about 4 m. In the middle of November it begins to snow, and thaws at the beginning or middle of May.

Station D: The air temperatures of plots at 500 and 800 m. in altitude of Mt. Waisuhorun have been calculated using the data of Kutchan Meteorological Station ($42^{\circ} 54' N$; 175.6 m. in altitude, about 12 km. E from Mt. Waisuhorun) with the lapse rate of $0.55^{\circ}/100$ m. The temperatures here closely resemble those of Korsakov in Sakharin ($46^{\circ} 39' N$; min. temp. -11.2° in Jan., max. temp. 17.2° in Aug., mean temp. 3.0°). Precipitation at Mt. Waisuhorun seems to be the same or somewhat higher than that of Kutchan, and the maximum snow depth is assumed to be 4-5 m. In the middle of November it begins to snow, and thaws at the middle or end of May.

The temperatures of these four stations appear to be the typical ones at the boundary of the cool temperate and the subarctic zones in Japan. The precipitations are also to be more or less similar, and may be enough or sometimes too much for the optimum plant growth under such low temperatures.

3. Soils

The chemical analyses of the soil were performed by the following treatments. The micro-Kjeldahl method was adopted for the determination of total nitrogen. Water-soluble elements in 10 g. of the air-dried soil were extracted with 50 ml. of Morgan's solution²⁶⁾ for 30 min. under shaking conditions. Nitrates and ammonium

Table 3. A comparison between observed and calculated air temperatures. The calculated values were obtained using mean lapse rate of $0.55^{\circ}/100$ m., for Ozegahara from the data observed at Yamaguchi and Minakami, and for Mt. Waisuhorun from those at Kutchan.

I. Station C (Ozegahara, 1450 m. in altitude)

	Monthly maximum		Monthly minimum	
	Observed*	Calculated	Observed*	Calculated
Jul. 1950	25.0°	23.8°	13.5°	13.1°
Aug. 1950	24.8	24.2	13.8	14.1

* cited from Daigo and Maruyama²⁵⁾

II. Station D (Waisuhorun, 550 m. in altitude)

	Daily maximum		Daily minimum	
	Observed	Calculated	Observed	Calculated
Apr. 12, 1959	4.8°	4.8°	-2.0°	-1.8°
Apr. 13	0.5	0.2	-2.7	-2.6
Jun. 2	12.0	11.9	9.5	9.6
Jun. 3	11.2	11.1	7.9	8.0
Aug. 8	23.8	24.0	12.1	11.6
Aug. 9	21.5	21.8	11.2	10.9
Oct. 11	13.1	12.6	3.3	3.4
Oct. 12	12.7	12.7	8.3	8.4
Oct. 13	15.8	15.8	4.0	3.9

nitrogen in the soil extract were determined by the diphenylamine method and the Nessler method, and for the measurements of total phosphorus and of calcium the ammonium-molybdate method and the calcium oxalate method were used, respectively.

Station A (Tomezuka): Under a litter layer about 7 cm. deep lies the A-horizon of a well weathered black loam of 60-80 cm. depth originated from volcanic deposits. Under this, there exists a layer of reddish brown clay loam of B-horizon. The roots and rhizomes of *Sasa* is mainly restricted within the A-horizon. Some of the results of chemical analyses of the A-horizon are shown in Table 4. It is clear that the soil is rich in humus, but is rather deficient in calcium and other available nutritional elements.

Station C (Ozegahara): The greater part of Ozegahara is covered with thick peat layers. There is, however, no pear layer on the forest ground along the streams running through the moor. The A-horizon of the forest soil on the streamside of the River Nujiri is a dark brown clay loam of about 7 cm. depth, and the B-horizon is a brown silt loam 60-70 cm. deep^{28,27)}, but sometimes it is pure sandy soil containing gravels.

Station D (Waisuhorun): The A-horizon consists chiefly of black or blackish brown loam about 35 cm. thick, and the distribution of rhizomes of *Sasa* is almost limited to the A-horizon. The B-horizon is a layer of wet clay loam reddish brown. As shown in Table 4, the A-horizon is rich in available nutritional elements, accumulating exchangeable calcium in a large amount in its surface layer.

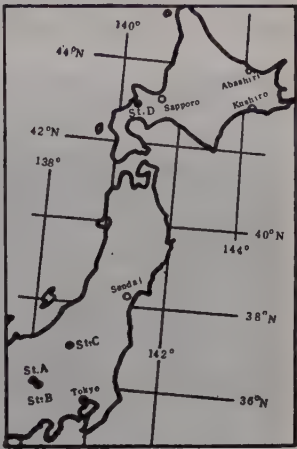


Fig. 1. Geographical locations of four stations. Station A: Tomezuka, Mt. Kirigamine. Station B: Mugikusa Pass, Mt. Yatsugatake. Station C: Ozegahara. Station D: Mt. Waisuhorun.

Table 4. Chemical characteristics of the soils at Tomezuka and Mt. Waisuhorun.

	Sampling depth (cm.)	Ignition loss (% of d.w.)	Total-N(%) (air-dried)	NH ₄ -N (mg. per 100 g. air dried soil)	NO ₃ -N	Ca	Total-P
St. A Tomezuka	3- 7	45.8	1.18	4.1	0.00	39.4	0.57
	18-20	44.7	1.06	4.1	0.08	25.6	0.59
St. D Waisuhorun	2- 5	47.2	1.23	37.5	1.62	123.3	1.84
	7-10	30.7	0.84	20.0	1.28	76.8	0.60
	15-20	20.4	0.54	8.0	0.79	51.3	1.54
	25-30	19.8	0.49	6.8	0.46	6.1	1.25

General Pattern of Reproduction and Estimation of Culm Ages

Sasa, evergreen perennial plant, does not reproduce generally by means of seeds which are rarely formed, but vegetatively by sprouting from rhizomes. The formation of buds occurs annually at the nodes of subterranean part of parent culms and of young rhizomes, and new culms develop on some of the rhizomes and new rhizomes develop on old rhizomes after dormant state for several years. Under field conditions mentioned above, the bud often sprouts out in a fairly distant place from the parent bush about a month after thawing of snow, and then new leaves develop.

Together with the growth of bud into a culm, the new rhizome continues to develop till November. In several years a few bush is completed by sprouting of buds which were mainly formed at the nodes around the culm.

The life span of a culm varies with species and environmental conditions. However, since the culm branches only once a year, we can assess the age of culm by the number of branching as illustrated in Fig. 2, though the estimation more than 4 years is not always easy because of the irregular branching occurring sometimes.

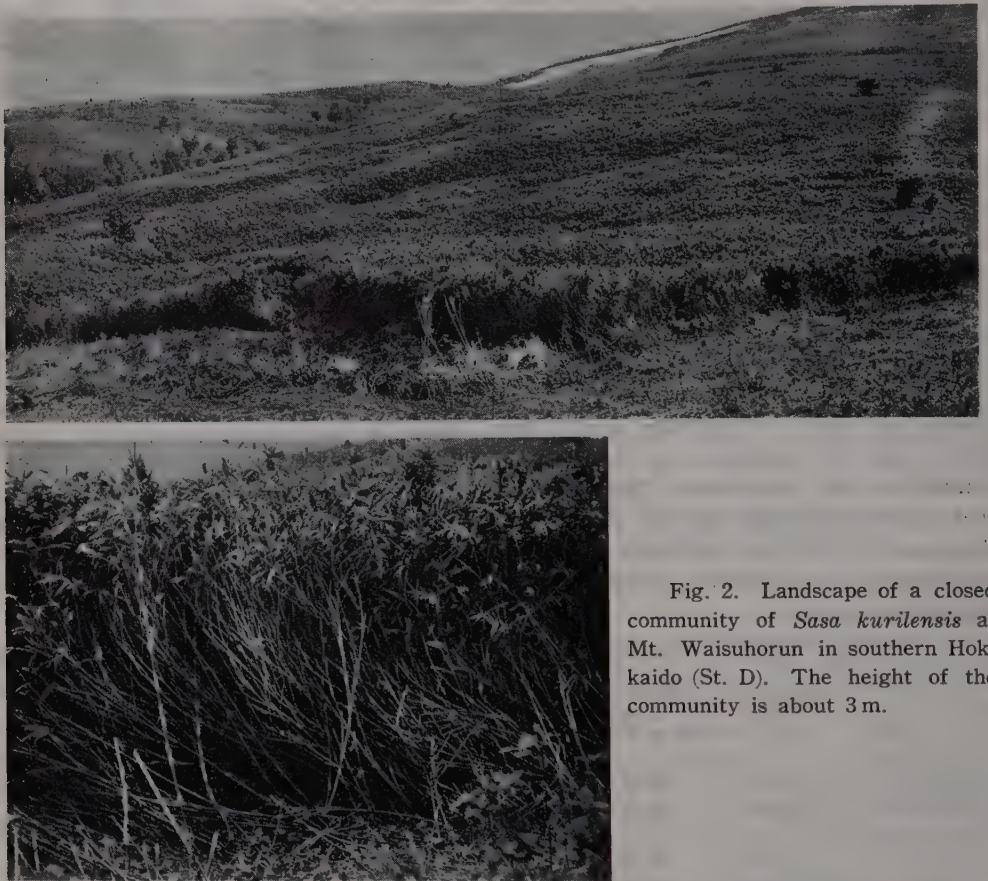


Fig. 2. Landscape of a closed community of *Sasa kurilensis* at Mt. Waisuhorun in southern Hokkaido (St. D). The height of the community is about 3 m.

The Standing Crop in Each Stand

Standing crop is an important amount for the estimation of growth of community under different environmental conditions and for the growth analysis of the community on the basis of dry matter production.

In order to decide the quadrat size to keep the sampling error under 10%, belt transects of $1 \times 25 \text{ m}^2$ were settled in the pure community of *Sasa* at each station. Basing on the results, a quadrat size of $50 \times 50 \text{ cm}^2$ was employed for Stations A, B, and C, and of $200 \times 200 \text{ cm}^2$ for Station D.

The estimation of standing crop was made in the following ways. The leaves, culms and subterranean parts of the whole *Sasa* plants within a quadrat were weighed in the fresh weight. Subsamples of each organ were measured in their dry weight

after drying up at 80° to estimate the standing crop in dry weight. The number of culms was also determined separately in their ages. In some cases, the weight of subterranean part was estimated from the ratio of the subterranean part to the aerial part, which is directly determined at the same station.

In Table 5, standing crops in summer including the subterranean part are given. The standing crops somewhat differ with stations. The smallest of ca. 1.4 kg./m.² was found at Mt. Kirigamine (Station A—Tomezuka) and the largest of 11.5 kg./m.² at Mt. Waisuhorum (Station D). These values were compared with standing crops of herb or grass communities in the montane grassland, lowland meadow and altherbosa in Japan^{4, 15, 23, 28-32}). Total dry weights at Stations A, B, and C resemble approximately those of the herb communities reported. On the contrary, the standing crop of *Sasa kurilensis* community at Station D is about twice as much as that of a *Cir-cium nipponicum* community at Ozegahara which is one of the largest values obtained in herb communities in Japan²³), and is about the same or somewhat larger in comparison with the standing crop of the community of the same *Sasa* species of Mt. Hakkoda reported by Yoshioka¹).

Large standing crops of *Sasa* communities depend chiefly on the huge amount of non-photosynthetic system, i.e. culms and rhizomes. The average life span of culms of *S. nipponica* at Station A is about two years, and that of *S. kurilensis* at Station D is 9.2 years. The interrelationships among the standing crop, the height of community and the longevity of culms at each station are illustrated in Fig. 3. The standing crop increases in proportion to the longevity of culms and the height of community, and decreases with decrease of population density (Table 5).

In spite of remarkable differences in standing crops among the four stations, there is recognized no marked difference in the weight of leaves per unit land area. Therefore, the ratio between non-photosynthetic and photosynthetic systems (C/F ratio after Iwaki¹⁴)) becomes larger with increase of standing crop (Table 5). This fact suggests that the fairly similar annual gross production may be expected in the *Sasa* communities with different dominant species.

Productive Structure of *Sasa* Communities

The growth rate of a plant community is naturally determined by the balance between the rates of anabolism and catabolism and moreover by the rate of shedding; these three rates are modified largely by the structure of the community, such as the vertical distribution of leaves, etc.^{4, 31, 32}), and environmental conditions. Accordingly, the growth of a plant community must be analysed as to structural and functional aspects of the community. Here the study of productive structure advanced by Monsi and Saeki⁴) is quite useful.

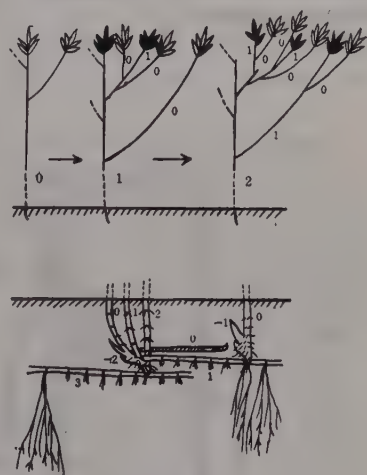


Fig. 3. Schematic figures showing the pattern of vegetative reproduction of *Sasa kurilensis*. Above: the aerial part. Below: the subterranean part. Numerals show the age classes, e.g. 0: the organs of the current year, 1: the organs 1 year old, -1: the organs that will come out in the next year. The age of a culm is indicated by the maximum number of branching.

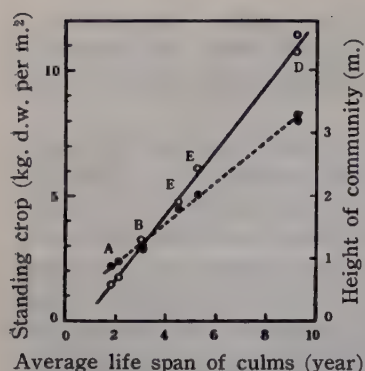


Fig. 4. Interrelationships between the standing crop per m^2 , the height of community, and the average life span of culms. A: *Sasa nipponica* (St. A, Tomezuka), B: *S. nikkoensis* (St. B, Mugikusa), D: *S. kurilensis* (St. D, Waisuhorun), E: *S. paniculata* (St. D, Waisuhorun 300 m. in altitude)

Representatives of the productive structures of *Sasa* communities of the four stations are illustrated in Fig. 5 after the stratifying clip method⁴). Relative light intensities in the communities are measured by the same method as reported in Hogetsu *et al.*¹⁹). The leaf area index, the total area of leaf surface per unit area of land surface, was calculated from the data of leaf dry weight in g. of $1 m^2$ stand and of leaf area in cm^2 of 1 g. dry weight of leaf (see Tables 5 and 6). Similar patterns of vertical distribution of leaves are found throughout these 4 diagrams irrespective of the difference in dominant species and localities, and they resemble well those of many terrestrial plant communities already reported^{4, 5, 8, 9, 12, 18, 15, 16, 18, 19, 23, 28, 31-35}).

Quantitative distribution in vertical direction of leaves determines the light intensities which have a direct influence on dry matter production of the plant community⁴). Light intensity I in a homogeneous community is reported by the formula⁴): $I = I_0 e^{-KF}$, where I_0 is the initial light intensity, F

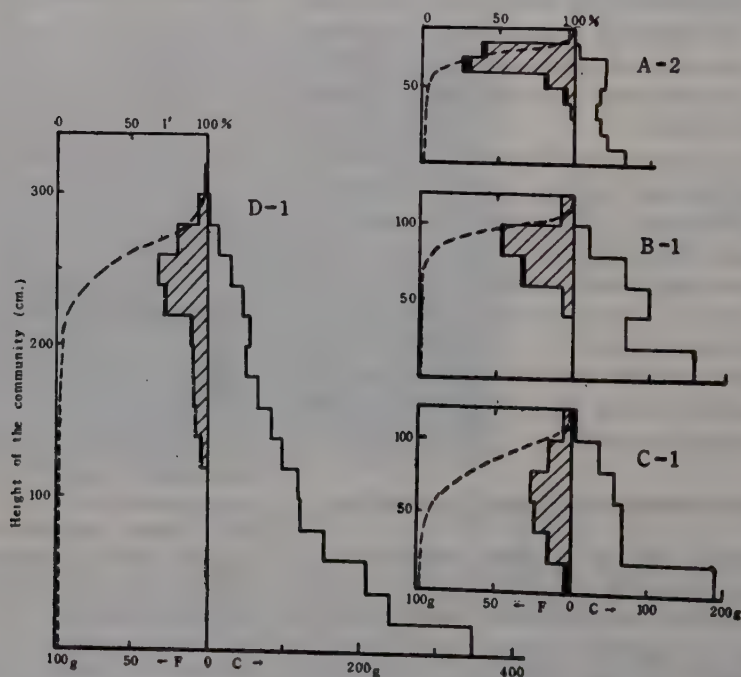


Fig. 5. Productive structure and relative light intensity in the *Sasa* community. Photosynthetic system (f) consists of laminae; non-photosynthetic system (c) of leaf sheaths, culms, etc. (fresh weight per $50 cm^2$). Black polygons mean the yellowing leaves. A: *Sasa nipponica* (St. A, Tomezuka), B: *S. nikkoensis* (St. B, Mugikusa), C: *S. oseana* (St. C, Ozegahara), D: *S. kurilensis* (St. D, Waisuhorun). See Table 5.

Table 5. Fresh and dry weight of whole plant, leaves, culms and subterranean parts per 1 m.² of *Sasa* communities at four stations in summer.
C/F-ratio=leaves (culms+subterranean parts) (in dry weight)

Station Species	A. Tomezuka <i>S. nipponica</i>		B. Mugikusa <i>S. nikkoensis</i>		C. Ozegahara <i>S. oseana</i>		D. Waisuhorun <i>S. kurilensis</i>	
Sampling No.	A-1	A-2	B-1	B-2	C-1	C-2	D-1	D-2
Sampling date	Jul. 14 1957	Aug. 27 1957	Aug. 13 1957	Aug. 13 1957	Jul. 10 1951	Aug. 6 1951	Aug. 8 1959	Aug. 15 1959
Height (cm.)	95	88	115	120	120	120	330	325
No. of culms	354	424	248	272	—	216	28	27
Leaves (g./m. ²)								
Fresh weight	664	617	729	693	711	561	915	874
Dry weight	276	256	330	318	317	250	475	456
Culms (g./m. ²)								
Fresh weight	1474	1194	3456	3371	3288	2392	13318	12448
Dry weight	605	490	1572	1574	1588	1158	7791	7282
Subterranean part (g./m. ²)								
Fresh weight	1885	1540	3683	3576	3469	2594	10060	9340
Dry weight	820	662	1326	1287	1313	974	3210	2984
Total (g./m. ²)								
Fresh weight	4023	3351	7868	7640	7468	5547	24293	22660
Dry weight	1701	1408	3228	3179	3218	2382	11476	10722
C/F-ratio	5.2	4.5	8.8	9.0	9.2	8.5	23.2	22.5

Table 6. Leaf area in cm.² per g. dry weight, light transmissibility of a leaf, leaf area index (*F*), and extinction coefficient (*K*) of *Sasa* species.

Species	Sampling No.	Leaf area	Transmissibility	<i>F</i>	<i>K</i>
<i>S. nipponica</i>	A-1	177	8.5- 9.8%	4.9	0.80
	A-2	177		4.5	0.72
<i>S. nikkoensis</i>	B-1	159	9.0-10.1	5.3	0.76
	B-2	159		5.0	0.80
<i>S. oseana</i>	C-1	163	10.6	5.2	0.80
	C-2	163		4.1	0.90
<i>S. kurilensis</i>	D-1	114	8.8- 9.9	5.4	0.70
	D-2	114		5.2	0.73

is the leaf area index, and *K*, the extinction coefficient.

The leaf area index in each stand fell in a narrow range of 4.5-5.4, except the slightly small value of 4.1 of a *Sasa oseana* community under an open deciduous broad-leaved forest at Station C-2. The extinction coefficient determined after the equation was 0.2-0.9 (see Table 6). The summer values, leaf area index 3.6-4.9, extinction coefficient 0.78, have already been reported for the community of *S. nipponica* on Mt. Kirigamine⁴⁾. The light transmissibility of a leaf is also one of the determinants for the extinction coefficient⁵⁾. The observed values in *Sasa* species were nearly 10% (cf. Table 6).

Light Intensity in *Sasa* Communities

Light intensities within a plant community, which are determined by the amount of leaves distributed above, play an important role in the fate of seedlings of various

species invading the community. The light factor in *Sasa* communities has a decisive influence upon the competition between *Sasa* and other plants, and consequently upon the continuity of the dominance of *Sasa* itself. The relative light intensities in *Sasa* communities, which were calculated by the equation with the values obtained of F and K should be 0.8–4.3% at the ground level homogeneously. In reality, however, the light intensities do not weaken homogeneously within a community. There are several sun flecks even under the most closed *Sasa* community. The frequency of sun flecks in the shade of communities where the light intensities are in general near the light minimum of seedlings, can influence upon their fate and consequently the development of the community.

The frequency distribution of light intensities under the *Sasa* communities was

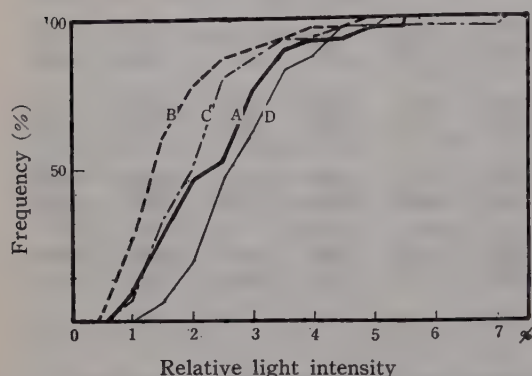


Fig. 6. Frequency curves of light intensity in the *Sasa* community. A: *Sasa nipponica* (St. A, Tomezuka), B': *S. nikkoensis* (St. B, Mugikusa), C': *S. oseana* (St. C, Ozegahara; under a forest of *Fagus crenata*-*Quercus crispula* near St. C.) and D: *S. kurilensis* (St. D, Waisuhorun).

from the result of a previous work⁶).

Such worse light conditions against seedling growth in the *Sasa* community seem to stay throughout the year, except for the snow season, due to the persistent evergreen leaves and elastic culms of *Sasa*. In winter, leaves and culms are buried under snow, being protected against the low temperature and severe dryness. With the progress of thawing in the spring, the rapid recovery into the former status of the community structure is made possible by the strong elasticity of culms. These characters must bring about the stability to the *Sasa* community in the plant succession. The detailed discussion on this problem concerning the function of community will be presented in other paper.

Summary

Many species of "Sasa" (bamboo grasses) are distributed endemically in Japan. They often constitute the characteristic grassland communities or the shrub strata of open forests in montane and subalpine zones (or cool temperate or subarctic zones). This study was carried out on closed communities of different dominant *Sasa* species at four stations of the central Honshu and of Hokkaido.

1. Descriptions as to temperatures, precipitations and soil conditions were given

investigated at Stations A, B, and D, and under a *S. oseana* community below a forest of *Fagus crenata* and *Quercus crispula* near Station C⁶). The investigated area was 30 m.² in each station. The area was divided into 1 m.² quadrats, and in every quadrat the relative light intensity on the ground was measured with a photocell at a point appointed by random numbers. The results obtained are illustrated in Fig. 6.

These frequency curves are more or less the same, and the frequency of relative light intensities lower than 5% is 97–100%. Under such low light conditions, it is probable that the dry matter production of seedlings of many species should be negative judging

as the basis of further discussion of *Sasa* communities.

2. The characteristics of the communities were investigated by the stratifying clip method in the weight of leaves, culms, subterranean parts and of entire plants, the height of community, the number of culms, the productive structure, the leaf area index, etc.

3. Among the communities with different dominant species and different localities, marked differences were realized in standing crop, height of community, longevity of culms, and number of culms. The standing crop increased with longevity of culms and height of community, and it is similar to or larger than the standing crop of other herb communities.

4. The amount and vertical distribution pattern of leaves of these *Sasa* communities were more or less the same. The leaf area index was 4.5-5.4, the extinction coefficient of light in the community, 0.7-0.9, and the light transmissibility of a leaf was about 10%.

5. On the ground surface of these *Sasa* communities, there prevailed extremely low light intensities with small deviation, enough to be fatal against seedlings of many species.

The author wishes to express his thanks to Prof. K. Hogetsu of Tokyo Metropolitan University and Prof. M. Monsi of the University of Tokyo for helpful suggestion and valuable advice. The author's deep gratitude is also due to Mr. Matsuoka and Mr. Tonouchi for kind help during the field survey of Station D.

References

- 1) Yoshioka, Y., *Ecol. Rev.* **5**: 117, 185, 304 (1939). 2) Ueda, K., and Uchimura, E., *Bull. Kyoto Univ. Forests* **27**: 112 (1958). 3) Boysen Jensen, P., *Die Stoffproduktion der Pflanzen*, Jena (1932).
- 4) Möller, C. M., *Forstl. Fors gsv., Danmark*, **17**: 1 (1944). 5) Monsi, M., and Saeki, T., **14**: Jap. J. Bot. **14** 22: (1953). 6) Kasanaga, H., and Monsi, M., *ibid.* **14**: 304 (1954). 7) Möller, C. M., Müller E., and Nielsen, J., *Forstl. Fors gsv., Danmark* **21**: 253, 273, 327 (1954). 8) Monsi, M., and Oshima, Y., *Jap. J. Bot.* **15**: 60 (1955). 10) —, Kunugi, R., and Kumekawa, A., *ibid.* **52**: 33 (1956). 11) Davidson, J. L., and Philip, J. R., *Climatol. and Microclimatol. UNESCO* 181 (1958).
- 12) Iwaki, H., *Jap. J. Bot.* **16**: 210 (1958). 13) —, *ibid.* **17**: 120 (1959). 14) Saeki, T., and Kuroiwa, S., *Bot. Mag. Tokyo* **72**: 27 (1959). 15) Midorikawa, B., *Ecol. Rev.* **15**: 83 (1959).
- 16) Saeki, T., *Bot. Mag. Tokyo* **73**: 55 (1960). 17) Kimura, M., *Misc. Rep. Res. Inst. Natur. Resour.* **52-53**: 36 (1960). 18) Kuroiwa, S., *Bot. Mag. Tokyo* **73**: 133, 165 (1960). 19) Hogetsu, K., Oshima, Y., Midorikawa, B., Sakamoto, M., Tezuka, Y., Mototani, I., and Kimura, M., *Jap. J. Bot.* **17**: 278 (1960). 20) Suzuki, T., *Sci. Res. Ozegahara Moor* 205 (1954). 21) Horikawa, Y., and Sasaki, Y., *ibid.* 288 (1954). 22) Yoshioka, K., *ibid.* 170 (1954). 23) Hogetsu, K., Ichimura, S., Hori, S., Oshima, Y., Kasanaga, H., Ono, H., and Takada, K., *ibid.* 313 (1954). 24) Oshima, Y., Kimura, M., Iwaki, H., and Kuroiwa, S., *Bot. Mag. Tokyo* **71**: 289 (1958). 25) Daigo, Y., and Maruyama, E., *Sci. Res. Ozegahara Moor*, 163 (1954). 26) Peech, T., and English, L., *Soil Sci.* **57**: 167 (1944). 27) Matsui, T., Kuwano, Yu., Kuwano, Yo., and Miyazawa, K., *Sci. Res. Ozegahara Moor* 78 (1954). 28) Kurasawa, H., and Sakamoto, M., *Misc. Rep. Res. Inst. Natur. Resour.* **40**: 81 (1956). 29) —, and —, *ibid.* **43-44**: 30 (1957). 30) Yamane, I., Ito, I., Sato, K., and Kamada, D., *Bull. Inst. Agr. Res. Tohoku Univ.* **8**: 227 (1957). 31) Kuroiwa, S., and Monsi, M., *J. Agr. Meteor.* **12**: 41 (1956). 32) Midorikawa, B., *Jap. J. Ecol.* **7**: 72 (1957). 33) Ichimura, S., *Jap. J. Bot.* **14**: 269 (1954). 34) Tezuka, Y., and Kusumoto, T., *Misc. Rep. Res. Inst. Natur. Resour.* **52-53**: 48 (1960). 35) Kuroiwa, S., *Bot. Mag. Tokyo* **72**: 413 (1959).

摘 要

大島康行： ササ群落の生態学的研究。I. 数種のササ群落の生産構造

ササ群落の生態学的研究の第一歩として、長野県霧ヶ峯のミヤコザサ、北八ツ岳 麦草峠のニッコウザサ、尾瀬ヶ原のオゼザサ、および北海道南部ニセコ火山群ワイヌホルン岳のチシマザサのよく発達した純群落の地域を調査地として選び、夏季に層別刈取り法を用いて、葉重、桿重、地下部重、全重、群落高、桿数、生産構造、葉面積指数などを測定した。またこれら四つの地域の気温、降水量、土壌条件の概況もあわせて検討した。

これらの四つの地域の種類を異にするササ群落の間では単位面積当たりの桿重、地下部重、現存量、桿数、および群落高、桿の平均寿命、および C/F 比はいちじるしく異なっている。現存量は桿の平均寿命が長く、群落高の高い種類ほど大きい。桿の平均寿命が 9.2 年のチシマザサ群落では最大の約 11 kg. d. w./m.² の現存量を示し、桿の平均寿命の最小の (1.8 年) ミヤコザサ群落では約 1.5 kg. d. w./m.² であった (図 4, 表 5)。日本の他の草原群落の現存量に比べて、前者はかなり大きく、後者はほぼ似た値であった。

一方、葉重、葉の垂直的分布の型は種類を異にする四つの群落の間でいちじるしい差はみられず、いずれも葉面積指数 4.5~5.4、群落の吸光係数 0.7~0.9、葉の透過率約 10% の範囲の値を示した (図 5, 表 6)。また主に葉によってきまる群落内相対照度も四つのササ群落の間でいちじるしい差はみられず、地表面近くの平均相対照度は 1~2% で低く、その変動の幅もきわめて小さいことが明らかにされた (図 6)。(東京都立大学理学部生物学教室)

Entwicklungsgeschichtliche Untersuchungen an den Characeen

I. *Nitella inokasiraensis*

von Naohiko IWASAKI*

Eingegangen am 28. Oktober, 1960

Die Characeen sind dafür bekannt, daß ihre Hauptachse aus langen einzelligen Internodien und mehrzelligen Knoten bestehen. Die Knoten, die den radiärweise ausgewachsenen Seitenorganen zugeordnet sind, wechseln mit den Internodien ab. Solche Beziehung zwischen beiden findet sich auch an den Blättern, den Seitensprossen und den Oogonien.

Die Characeen-Pflanzen wurden schon von zahlreichen Autoren an den verschiedenen Arten morphologisch und zytologisch untersucht. Diese Forscher sind Sachs¹⁾, Giesenhausen²⁻⁵⁾, Ernst^{6,7)}, Kuczewski⁸⁾, Goebel⁹⁻¹¹⁾, Bessenich¹²⁾, Drew¹³⁾, Walther¹⁴⁾, Sundaralingam¹⁵⁾ u.a. Sie widmeten sich den Untersuchungen der Zellteilung und der Fortpflanzungsorgane, infolgedessen gaben sie uns Einzelheiten der entwicklungsgeschichtlichen Morphologie an einzelnen Arten. Dabei handelte es sich um die strenge Regelmäßigkeit der Beziehungen zwischen den Zellen.

Die typischen Arten jeder Gruppe wählte ich aus den Characeen Japans aus, um die Entwicklungsgeschichte des Vegetationspunktes und der Geschlechtsorgane anatomisch-entwicklungsgeschichtlich zu untersuchen.

Die kleine Gruppe der Monoarthrodactylae, zu denen *Nitella inokasiraensis* gehört, hat die morphologische Eigenschaft, daß die Blätter meist zu sechs im Quirl angeordnet sind, wobei sie oft noch zwei Seitenblättchen, manchmal auch noch zwei Seitensprosse hat¹⁶⁾. Für die Monoarthrodactylae wurden folgende Arten schon anatomisch oder entwicklungsgeschichtlich erklärt: Sachs¹⁾ schrieb über den Vegetationspunkt und die beiden Geschlechtsorgane von *Nitella flexilis*; Giesenhausen³⁾, über den Bau der Sproßknoten von *N. syncarpa*; Ernst⁶⁾, über die Mißbildungen der Oogonien von *N. syncarpa*; Drew¹³⁾, über das Blatt und den adventiven Sproß von *N. opaca*; und schließlich Walther¹⁴⁾, über die Entwicklung der Geschlechtsorgane und andere zytologische Fragen von *N. syncarpa*.

Material und Methode.

Nitella inokasiraensis wurde im Frühling des Jahres 1958 aus einem kleinen Bach, der aus dem Teich des Inokasira-Parkplatzes in Tokyo strömt, gesammelt. Das Material wurde mit dem Formalin-Alkohol-Essigsäuregemisch fixiert, nach der üblichen Weise durch Butanol als Intermedium in Paraffin übergeführt. Die Schnittdicke betrug 10 μ . Das Präparat wurde mit Delafields Hämatoxylin gefärbt.

Entwicklung des Hauptsprosses.

Wie es bei anderen Characeen-Pflanzen der Fall ist, hat *Nitella inokasiraensis* auch die Eigenart, daß an ihrer Hauptachse die Knoten und Internodien miteinander abwechseln. Der Sproß stellt ein unbegrenztes Spitzenwachstum dar. Der Vegeta-

* Biologisches Institut der Städtischen Universität zu Tokyo, Tokyo, Japan.

tionspunkt ist eine halbkugelförmige Scheitelzelle (Abb. 1a). Nach der Längsstreckung teilt sich die Scheitelzelle durch eine horizontale Wand in eine neue Scheitelzelle und eine daruntergelegene scheibenförmige Zelle, die nach Giesenhagen⁸⁾ die Gliederzelle

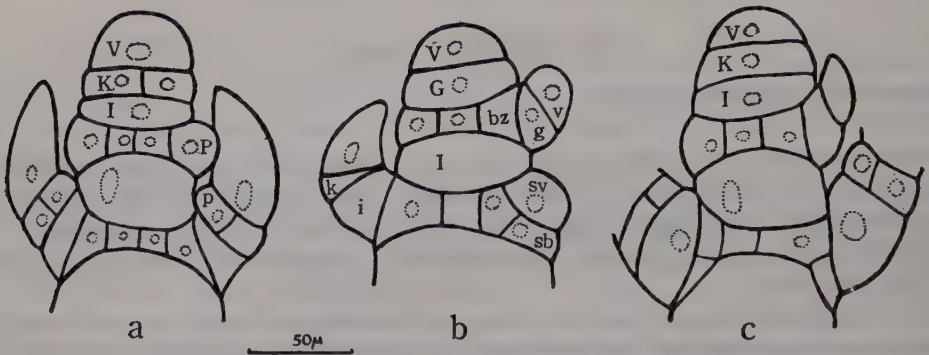


Abb. 1a-c. Mediane Längsschnitte der Sproßspitze bei *Nitella inokasiraensis* in drei aufeinanderfolgenden Entwicklungsstadien. bz, Basalzelle des Blattes; G, Gliederzelle des Hauptsprosses; g, Gliederzelle des Blattes; I, Internodium des Hauptsprosses; i, Internodium des Blattes; K, Knoten des Hauptsprosses; k, Knoten des Blattes; P, periphere Zelle des Sproßknotens; p, periphere Zelle des Blattknotens; sb, Urzelle des Seitenblättchens; sv, Scheitel des Seitensprosses; V, Scheitelzelle des Hauptsprosses; v, Scheitelzelle des Blattes.

ist (Abb. 1b). Bevor die neue Scheitelzelle zur ursprünglichen Größe heranwächst und sich zu teilen beginnt, erfolgt eine horizontale Teilung in der Gliederzelle, dadurch die zwei übereinanderliegenden Zellen hervorgebracht werden. Die Teilungswand

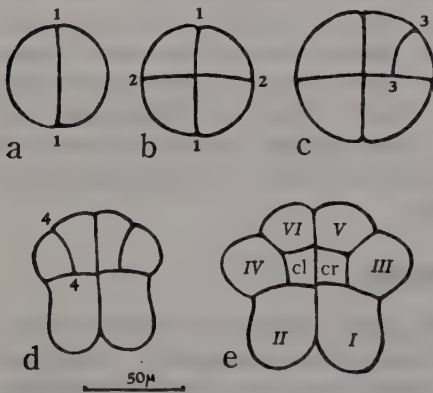


Abb. 2a-e. Querschnitte junger Sproßknoten von *Nitella inokasiraensis* in verschiedenen Stadien der Zerlegung in die peripherischen Zellen und die beiden Zentralzellen. I-VI, periphere Zellen; cl, cr, Linke und rechte Zentralzellen.

denen ist die Zelle unten rechts die erste periphere Zelle und die Zelle unten links die zweite³⁾. Aus den anderen Zellen gehen noch vier periphere Zellen und zwei Zentralzellen hervor. Die dritte periphere Zelle tritt in der rechten Seite

wölbt sich nach oben etwas hervor. Die obere Zelle, die konkav-linsenförmig ist, ist die Urzelle des Sproßknotens, und die untere bikonvex-linsenförmige Zelle wird direkt zum Internodium, ohne sich weiter zu teilen (Abb. 1c). Diese Internodialzelle streckt sich außergewöhnlicherweise in die Länge. Die Urzelle des Knotens erfährt eine Reihe von gesetzmäßigen Teilungen, dadurch die Anlagen der Blätter, der Seitensprosse und der Seitenblättchen entstehen.

Die Knotenzelle teilt sich längsweise in ihrer Mitte in zwei nebeneinanderliegende gleichgroße Zellen, die von Giesenhagen⁸⁾ Halbierungszellen genannt wurden (Abb. 2a). Die zweite Längsteilung, die die erste senkrecht kreuzt, folgt in den Halbierungszellen, so daß vier gleichmäßige Zellen erzeugt werden (Abb. 2b). Unter

als ein kleines Stück neben der ersten peripherischen Zelle auf, welches von oben gesehen, wie eine fächerförmige Zelle aussieht (Abb. 2c). Ein wenig später bildet sich die vierte peripherische Zelle auf der linken Seite, die gegen die dritte symmetrisch liegt (Abb. 2d). Die fünfte wird zwischen der dritten und der Halbierungswand angelegt, und die sechste zwischen der vierten und der fünften (Abb. 2e). Infolgedessen umschließt der Ring der sechs peripherischen Zellen zwei Zentralzellen, die noch weiter teilungsfähig sind.

Entwicklung der Blätter.

Die dritte bis sechste peripherische Zellen sind kleiner als die erste und zweite. Jede dieser vier peripherischen Zellen wölbt sich zuerst nach oben schräg, dann schneidet sie eine Basalzelle nach unten durch eine zum Umfang des Knotens parallele Wand ab (Abb. 4A). Die oben gelegene Zelle ist die Anlage des Blattes; sie ist in ihrer Teilung der Scheitelzelle des Hauptsprosses ähnlich. Zuerst trennt sie unterwärts eine Gliederzelle, welche dann sich in eine Knoten- und eine Internodialzelle teilt (Abb. 1b). Der Scheitel selbst streckt sich, ohne sich weiter zu teilen, läuft spitz zu und wird danach direkt zu einem Blattendglied.

Die Knotenzelle des Blattes unterscheidet sich von dieser des Hauptsprosses darin, daß sie kleiner als die letztere ist, und daß sie direkt ihre peripherischen Zellen abschneidet, ohne sich in Halbierungszellen teilend. Die erste peripherische Zelle trennt sich von der Knotenzelle auf der dem Hauptsprosse zugekehrten Seite mit einer nach innen gewölbten Wand ab (Abb. 3a, 1-1). Nach der Trennung wächst die erste peripherische Zelle nach oben schräg, spitzt sich und wird zu einem Blattendglied. Die folgenden vier peripherischen Zellen scheiden sich von der Knotenzelle wechselnd links und rechts, und gleichzeitig abaxialwärts ab, so daß eine Zentralzelle von fünf peripherischen Zellen in der Mitte eingeschlossen wird (Abb. 3b, c). Die Zentralzelle ist noch weiter teilungsfähig.

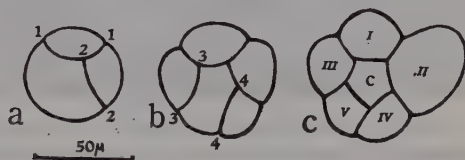


Abb. 3a-c. Querschnitte junger Blattknoten von *Nitella inokasiraensis*. I-V, peripherische Zellen des Blattes.

Die erste und zweite peripherische Zelle, und unter Umständen auch die dritte, werden zu den Blattendgliedern. Also, einschließlich des mittleren Blattendgliedes stehen immer drei oder vier Blattendglieder auf einem Blattknoten. Die zurückbleibenden peripherischen Zellen, d.h. die vierte und fünfte, eventuell die dritte, strecken sich nicht, sondern teilen sich in einige kleine Zellen, die danach den Blattknoten mit den Zentralzellen gestalten.

Entwicklung der Basalzelle der Blätter.

Die Basalzelle der dritten bis sechsten Blätter teilt sich in gleicher Weise, indessen gibt es dabei verschiedene Abweichungen. Zuerst tritt eine Querwand auf, die senkrecht zur Längsachse der Zelle liegt. Die zwei übereinanderliegenden Zellen sind ungefähr von gleicher Größe. Dann werden die beiden Zellen durch eine mediane Längswand in je zwei gleichwertige Zellen geteilt. Bis zu diesem Stadium ist die Teilung bei den vier Basalzellen gleich. Weitere Teilungen verlaufen ohne besondere Regelmäßigkeiten. In den Basalzellen eines erwachsenen Sproßknotens finden sich manchmal viele kleine Zellen. Unter den günstigen Bedingungen entstehen aus diesen

Zellen adventive Sprosse auf der adaxialen Seiten der Blattbasis und Rhizoiden auf den ad- und abaxialen Seiten.

Entwicklung der ersten und zweiten peripherischen Zellen sowie Entstehung des Seitensprosses.

Die erste und zweite peripherische Zellen, die im weiteren Verlauf der Zellteilung von den anderen abweichen, entwickeln sich den anderen vorangehend. Aus den beiden ersteren Zellen kommen zwei Blätter, zwei Seitensprosse und zwei Seitenblättchen vor.

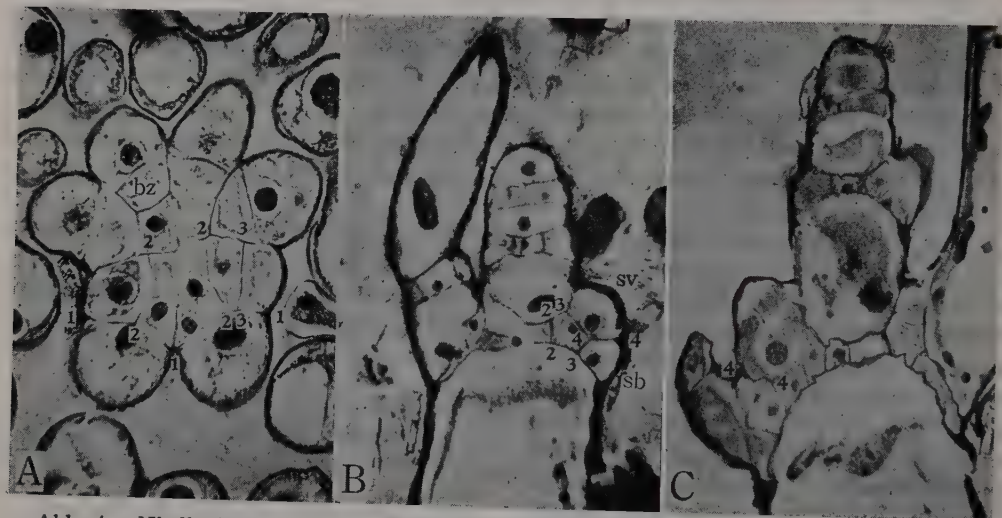


Abb. 4. *Nitella inokasiraensis*. A, Querschnitt des Sproßknotens. B, C, Mediane Längsschnitte durch die Anlage eines Seitensprosses bzw. eines Seitenblättchens am Sproßknoten. 1-1, 2-2,, aufeinanderfolgende Teilungswände; bz, Basalzelle des Blattes; sb, Urzelle des Seitenblättchens; sv, Scheitel des Seitensprosses.

Die ersteren peripherischen Zellen wölben sich zuerst nach oben schräg hervor, dann teilen sie sich durch eine Wand, die in der Abb. 4A mit 1-1 bezeichnet ist. Diese Teilungswand durchzieht die Basis der Vorwölbung, so daß die Basalzelle und die Anlage des Blattes abgetrennt werden. Auf der Basalzelle bleibt daher ein freier Oberfläche zwischen der Basis der Blattanlage und benachbarten peripherischen Zelle. Die Anlagen der beiden ersteren Blätter entwickeln sich in gleicher Weise wie die Anlagen der dritten bis sechsten Blätter.

Die Basalzelle wölbt sich an der Stelle der freien Oberfläche hervor. Dann teilt sie sich an der Basis des vorgewölbten Teils (Abb. 4A, 2-2). Diese 2-2 Teilungswand liegt ungefähr parallel zur Halbierungswand. Die dadurch hervorgebrachte innen eingeschlossene Basalzelle teilt sich danach unregelmäßig, um kleine Zellen an den Basen des ersten und zweiten Blattes zu erzeugen.

Im nach außen vorgewölbten Teil tritt die 3-3 Wand parallel zur 2-2 Wand auf (Abb. 4A). Die innere Zelle wird zur Basis des Seitensprosses. Die äußere Zelle wölbt sich weiter nach außen, danach teilt sie sich quer in der Mitte in zwei gleichmäßige Zellen, die übereinander liegen (Abb. 1b, 4B). Die obere Zelle ist der Scheitel des Seitensprosses, und untere wird zur Urzelle des Seitenblättchens. Der Scheitel des Seitensprosses vergrößert sich zunächst, und dann teilt eine Gliederzelle.

ab, wie der Scheitel des Hauptsprosses. Die Gliederzelle teilt sich in eine Knoten- und eine Internodialzelle. Danach folgen in der Knotenzelle die regelmäßigen Teilungen, die mit diesen des Hauptsprosses übereinstimmen. Der so entstandene Seitensproß wächst sich unter geeigneten Bedingungen aus.

Die Urzelle des Seitenblättchens streckt sich zuerst waagrecht nach außen, dann teilt sie sich in eine Basalzelle und eine Blattanlage. Weitere Teilungen sind denen der ersten bis sechsten Blätter gleich, außer daß Seitenblättchen vorwiegend kleiner als die letzteren Blätter ist (Abb. 4C).

Die Basalzellen der Seitenblättchen teilen sich weiter, und kommen kleine Zellen vor. Aus diesen kleinen Zellen und den gleichmäßigen Zellen an den Basen der beiden ersteren Blätter entwickeln sich adventive Sprosse und Rhizoiden, wie es bei den Basalzellen der dritten bis sechsten Blätter der Fall ist.

Entwicklung der Geschlechtsorgane.

Nitella inokasiraensis ist diözisch, sie entwickelt ihre Antheridien oder Ooognien im Frühling auf den Blattknoten.

Entwicklung des Antheridiums: In der reproduktiven Phase teilt sich die Blattanlage der männlichen Pflanzen wie die der vegetativen Pflanzen. Der so hervorgebrachte Blattscheitel spitzt sich nicht, sondern rundet sich, dann trennt eine scheibenförmige Zelle nach unten (Abb. 5a). Diese abgetrennte Zelle erfährt später noch eine horizon-

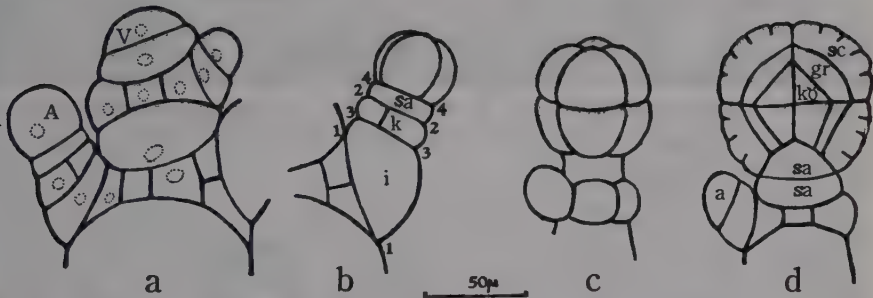


Abb. 5. a, Längsschnitt der männlichen Pflanzen von *Nitella inokasiraensis*. b-d, Entwicklung des jungen Antheridiums in verschiedenen Stadien. A, Anlage des Antheridiums der ersten Ordnung; a, Anlage des Antheridiums der zweiten Ordnung; gr, Griffzelle; i, Blattinternodium; k, Blattknoten; kö, Köpfchenzelle; sa, Stiel des Antheridiums; sc, Schildzelle; V, Scheitelzelle des Hauptsprosses.

tale Teilung, wodurch die zwei übereinanderliegenden Zellen des Stiels vom Antheridium erzeugt werden (Abb. 5d). Die oben gelegene kugelförmige Scheitelzelle wird durch zwei senkrecht kreuzende Längswände in vier gleichmäßige Teile geteilt (Abb. 5b). Die dritte Teilungswand ist horizontal, dadurch acht gleichmäßige Zellen aus einer Anlage hervorgebracht werden (Abb. 5c). In den Zellen treffen zwei zur Oberfläche parallel laufende Wände aufeinander. Die äußersten Teile werden zu den Schildzellen, die mittleren zu den Griffzellen und die innersten zu den Köpfchenzellen (Abb. 5d). Die spermatogenen Fäden gehen von den Köpfchenzellen aus (Abb. 6B).

Zum Blattknoten unter dem Antheridium gehören fünf periphere Zellen. Ein bis zwei von denen können die Antheridien der zweiten Ordnung bilden, wobei eine Basalzelle dieses Antheridiums am Blattknoten auftritt (Abb. 6A). Die Entwicklung der Antheridien der zweiten Ordnung sind dem von der ersten Ordnung gleich. Die

anderen peripherischen Zellen werden meistens zu den Blattendgliedern, die sonstigen peripherischen Zellen strecken sich nicht, sondern teilen sich danach.

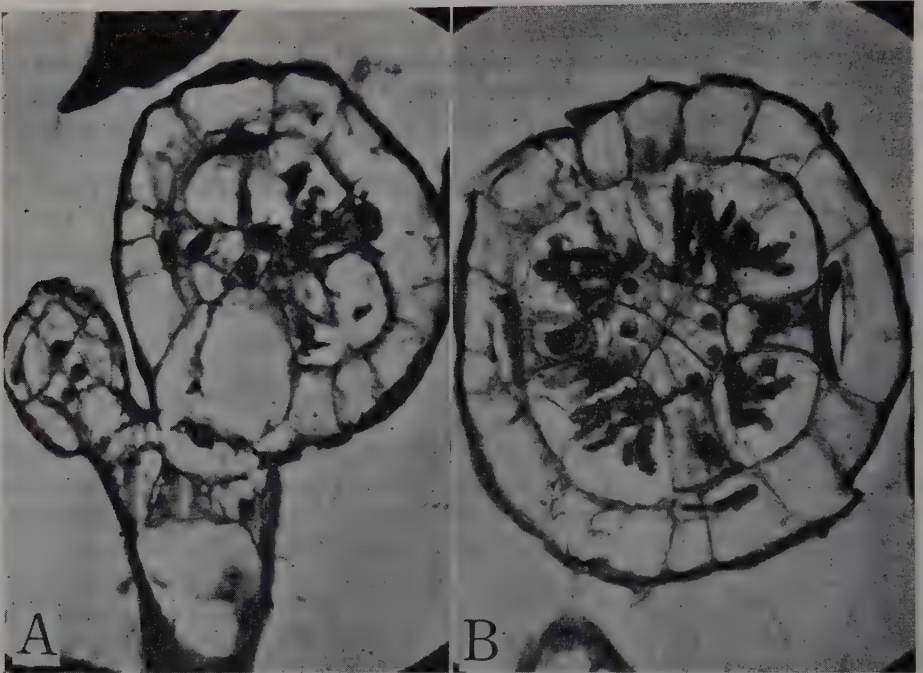


Abb. 6. *Nitella inokasiraensis*. A, Längsschnitt des Antheridiums der ersten und der zweiten Ordnung. B, Querschnitt des Antheridiums, spermatogene Fäden schon aus den Köpfchenzellen ausgegangen.

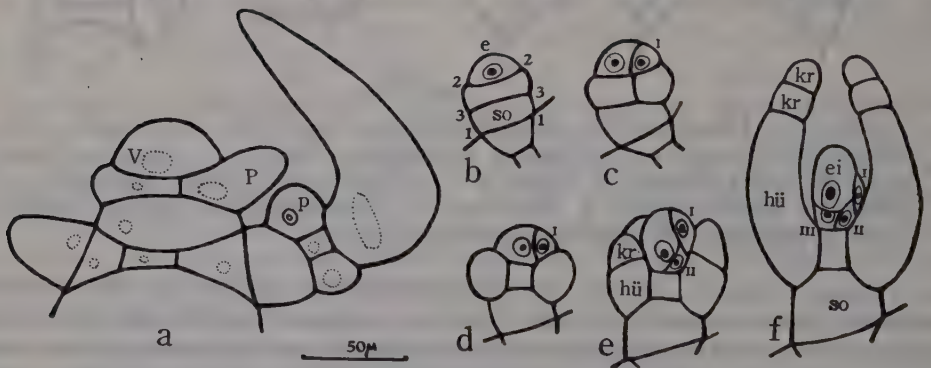


Abb. 7. a, Längsschnitt der weiblichen Pflanzen von *Nitella inokasiraensis*. b-f, Entwicklung des Oogoniums in verschiedenen Stadien. e, Eimutterzelle; ei, Eizelle; hü, Hüllschläuchen; kr, Krönchenzelle; P, periphere Zelle des Sproßknotens; p, periphere Zelle des Blattes; so, Stiel des Oogoniums; V, Scheitelzelle des Hauptsprosses.

Entwicklung des Oogoniums: Die weiblichen Pflanzen entwickeln sich wie die vegetativen Pflanzen (Abb. 7a). Ein bis drei von den fünf peripherischen Zellen des Blattknotens wölben sich zuerst hervor, dann teilen sie sich in zwei mit einer parallel zum Knotenumfang laufenden Wand. Die innere Zelle wird zur Basis des Oogoniums.

Die äußerere Zelle, die nichts anderes als die Anlage des Oogoniums, schwillt weiter und rundet sich ab. Diese Anlage verhält sich wie eine Scheitelzelle. Zuerst teilt sie sich mit einer Wand, die parallel zur vorangehenden Wand gelegen wird. Die kuppel-kugelförmige Scheitelzelle ist die Eimutterzelle, und die darunter gelegene Gliederzelle teilt sich quer in eine Knoten- und eine Internodialzelle (Abb. 7b). Die Internodialzelle wird zum Stiel des Oogoniums. Die Knotenzelle teilt sich in fünf peripherische Zellen und eine Zentralzelle. Die Zentralzelle erfährt danach keine Teilung. Die peripherischen Zellen werden zu den Hüllschläuchen; dabei strecken sie sich, und treten zweimalige Teilungen an ihren Spitzen auf. So entstehen die oberen und unteren Krönchenzellen (Abb. 7f).

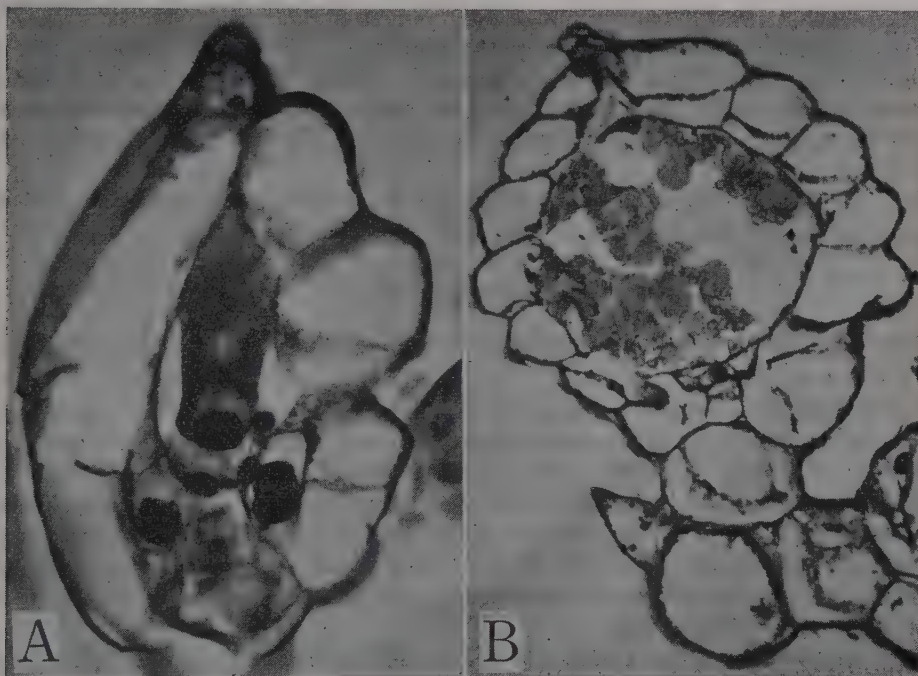


Abb. 8. *Nitella inokasiraensis*. Längsschnitte des Oogoniums. A, junges Oogonium, Eizelle und drei Wendezellen sichtbar. B, älteres Oogonium, schon Material gespeichert.

Die Eimutterzelle schneidet folgendermaßen die drei Wendezellen¹⁴⁾ ab; die erste Wendezelle wird zu der Zeit, wo die erste peripherische Zelle des unteren Knotens entsteht, gerade auf der ersten durch einer Längswand als eine kleine Zelle seitlich abgetrennt (Abb. 7c, d). Die zweite Wendezelle wird aus dem unteren Teil der Eimutterzelle neben der ersten durch eine Längswand abgeschnitten (Abb. 7e). Die dritte Wendezelle wird aus der Eimutterzelle parallel zur Grundfläche geschnitten (Abb. 7f, 8A). Die obere größere Zelle ist die Eizelle, die sich danach vergrößert und viel Material speichert (Abb. 8B). Dagegen bleiben die drei Wendezellen in ihrer anfänglichen Größe zurück.

Besprechung.

Die Gliederzelle, die aus der Scheitelzelle geschnitten wird und in der Folge zur Knoten- und Internodialzelle wird, ist an verschiedenen Orten vorhanden, d.h. am

Haupt- und Seitensproß, an der Anlage des Blattes, des Seitenblättchens und des Oogoniums. Derartige Teilungsvorgänge sind von den anderen Arten her bekannt. Das zeigt schon Giesenhausen³⁾ in einer Formel; $V=v+g=v+(k+i)$. Meiner Meinung nach ist sie richtig.

Die Basalzelle ist eigentlich eine Gliederzelle. Bei den Charen und *Nitellopsis* teilt sich die Basalzelle in eine Knoten- und eine Internodiazelle. Bei *Nitella inokasiraensis* erfährt die Gliederzelle keine Knoten-Internodium-Teilung, sondern direkt die Entwicklung der Basalzelle. Bei *Nitella hyalina* nach Ernst⁷⁾ gibt es auch keine Knoten-Internodium-Teilung, an *N. pseudoflabellata* nach meiner unveröffentlichten Beobachtung auch nicht. Das stimmt mit der Beobachtung von Giesenhausen^{3,4)} an *N. gracilis*, *N. syncarpa* und *N. cernua* überein. Das mag eine Besonderheit der Nitellen sein.

Zum Knoten, der aus der Gliederzelle vorkommt, gehören der Sproßknoten, der Blattknoten und der Knoten des Oogoniums. Bei den schon untersuchten Arten der Characeen ist es üblich, daß die erste Teilung des Sprosses die Halbierung ist, und daß keine Halbierungswand im Blattknoten auftritt. Bei *N. inokasiraensis* ist es auch so.

Bei der Entwicklung der Eimutterzelle von *N. inokasiraensis* entstehen die drei Wendezellen, wie es bei der von *N. syncarpa*¹⁴⁾ der Fall ist.

Zusammenfassung.

Nitella inokasiraensis, die zu den Monoarthrodactylae gehört, wurde entwicklungsgeschichtlich untersucht.

Der Hauptsproß besitzt ein unbegrenztes Spitzenwachstum. Der Vegetationspunkt ist eine halbkugelförmige Scheitelzelle, die unterwärts eine Gliederzelle trennt. Die Gliederzelle teilt sich in eine Knoten- und Internodiazelle, die sich danach ohne Zellteilung streckt. Die Knotenzelle teilt sich zuerst in zwei nebeneinanderliegende Halbierungszellen, dann erfährt sie eine Reihe von gesetzmäßigen Teilungen, aus der die sechs peripherischen Zellen und die zwei Zentralzellen entstehen. Jede von den peripherischen Zellen wölbt sich zuerst nach oben schräg, dann teilt sie sich in eine Basalzelle und eine Blattanlage.

Aus den Basalzellen des ersten und zweiten Blattes kommen die zwei Seitensprosse und die zwei Seitenblättchen vor.

Bei *Nitella inokasiraensis* erfährt die Basalzelle keine Knoten-Internodium-Teilung.

Nitella inokasiraensis ist diözisch, sie entwickelt ihre Antheridien oder Oogonien auf den Blattknoten.

Die Eimutterzelle schneidet die drei Wendezellen außer der Eizelle ab.

Es ist mir eine angenehme Pflicht, an dieser Stelle Herrn Prof. Dr. H. Ono und Herrn Prof. H. Kasaki für ihre Anregungen und stetige Leitung meinen besten Dank auszusprechen.

Literaturverzeichnis.

- 1) Sachs, J., A Text-Book of Botany (Engl. Transl. by S. H. Vines), 292 (1882). 2) Giesenhausen, K., Flora **82**: 381 (1896). 3) —, ibid. **83**: 160 (1897). 4) —, ibid. **85**: 19 (1898). 5) —, Ber. deutsch. Bot. Ges. **19**: 277 (1901). 6) Ernst, A., Flora **88**: 1 (1901). 7) —, Vierteljahrschr. Naturf. Ges. Zürich **49**: 64 (1904). 8) Kuczewski, O., Beihefte Bot. Centralbl. **20**, Abt. I: 25 (1906). 9) Goebel, K., Flora **90**: 279 (1902). 10) —, ibid. **110**: 344 (1918). 11) —, ibid. **124**: 491 (1930). 12) Bessenich, K., Jahrb. f. wiss. Bot. **62**: 214 (1923). 13) Drew, K. M., Ann.

Bot. **40**: 321 (1926). 14) Walther, E., Arch. Jul. Klaus-Stiftung. **4**: 23 (1929) 15) Sundaralingam, V. S., Jour. Ind. Bot. Soc. **33**: 272 (1954). 16) Migula, W., Paschers Süßwasser-Flora Deutschlands, Österreichs und der Schweiz. Heft **11**: 207 (1925).

摘 要

岩崎尚彦: ジャジクモ科植物の生長点の分化と器官形成. I. *Nitella inokasiraensis*.

単節類に属する *Nitella inokasiraensis* イノカンラフラスコモの形態形成を観察した.

主軸は無限生長を行なう. 生長点は 1 個の頂端細胞で, これが下に 1 個の細胞を分裂する. その細胞がさらに節の原基と節間細胞に分裂する. 節間細胞は以後細胞分裂を行わず伸長する. 節の原基は最初左右 2 等分されたのち, 規則的に分裂し, 6 個の周辺細胞と 2 個の中心細胞をつくる. 各周辺細胞は斜上方に伸長してから, 基底細胞と小枝原基に分裂する.

第 1・2 小枝の基底細胞から 2 個の腋芽と 2 本の二次小枝ができる.

Nitella inokasiraensis の基底細胞は節と節間に分裂しない.

Nitella inokasiraensis は雌雄異株で, 小枝節に藏精子または藏卵器をつける.

卵母細胞からは卵細胞のほかにも 3 個の細胞ができる. (東京都立大学理学部生物学教室)

On Two New Species of *Spirillum*

by Yasuke TERASAKI*

Received November 4, 1960

The present author reported on the isolation and the taxonomic studies of a new species of *Spirillum*, *Spirillum putridiconchylum*¹⁾. Thereafter he isolated new species of *Spirillum* from the putrid bodies of two species of the fresh water shell fishes, *Corbicula japonica* Prime and *Cipangopaludina malleata* (Reeve). This paper deals with the isolation and the morphological, cultural and physiological studies of these spirilla.

Isolation

Source of materials: *Corbicula japonica* Prime used in this study was purchased at the market at Takanobashi in Hiroshima city in October 1958; *Cipangopaludina malleata* (Reeve) was collected at the paddy-field at Kairoshiohama in Itsukaichi-cho, near Hiroshima city in May 1959. The putrid media in which the spirilla had developed were obtained by the same procedure as mentioned in a previous paper¹⁾. Among several organisms which were isolated by plating on the putrid media, the following two spirilla were identified as the new species *Spirillum metamorphum* nov. sp. (isolated from the putrid medium of *Corbicula japonica* Prime) and *Spirillum crassum* nov. sp. (from the putrid medium of *Cipangopaludina malleata* Reeve).

Method and Media: Method for the isolation is the same as in *Spirillum putridiconchylum*¹⁾. But two media employed in this study were as follows. a) For *Spirillum metamorphum*; peptone 5 g., yeast extract 3 g., sodium chloride 1 g., shell fish extract 200 ml., water 800 ml. Preparation of the liquid media and the solid media is the same as that mentioned previously¹⁾ except the sort of shell fish used to obtain the extract, to say, in this case *Corbicula japonica* Prime was used. b) For *Spirillum crassum*; peptone 5 g., beef extract 3 g., yeast extract 3 g., water 1000 ml. For preparing the solid media, 0.7% (w/v), agar powder was added. The pH of media was adjusted to 7.0 to 7.2 before sterilizing.

Culture media and cultural conditions for the identification of the spirilla

Culture media and cultural methods for the identification of the spirilla were the same as used for the identification of *Spirillum putridiconchylum*¹⁾, but the pH of the culture media and the temperature of incubation were as follows. The pH of the media was adjusted to 8.0 to 8.2 for *Spirillum metamorphum*, and 7.4 to 7.6 for *Spirillum crassum*; the temperature of incubation was 30° for *Spirillum metamorphum*, and 38 to 40° for *Spirillum crassum*. The gelatine plate and stab were incubated at 20° for the both species.

Descriptions of the species *Spirillum metamorphum* nov. sp.

1. Morphological characteristics

Vegetative cell: The initial cultures of the spirillum contain the definite spiral

* Biological laboratory, Suzugamine Women's College, Hiroshima Japan.

cells in high percentage, but the repeated subcultures scarcely contain the definite spiral cells. The spirillum grown in the nutrient broth after incubation for 24 hrs. has a rod- or a vibrio-shaped cell which is 2 to 12 microns in length, or a S- or a spiral-shaped cell which is 10 to 20 microns in length, with obtuse ends. In the spiral-shaped cell, the wave length is 10 to 12 microns and the width of the wave is 2 to 3 microns. The S- or the spiral-shaped cell often appears to be composed of two or more cells. The diameter of the cell is 1.2 to 1.4 microns. Refractive granules are seen in some individuals but not in others. The motility of the spirillum is screwlike in the rod- or the vibrio-shaped cell as well as in the S- or the spiral-shaped cell. In old culture, the vegetative cells predominate and the microcysts are not found. The cell taken from a colony on the nutrient agar plate after 24 hrs. is a rod- or a vibrio-shaped cell of 2 to 6 microns in length. The diameter of the cell is almost the same as that of the cell grown in the nutrient broth. Concerning the refractive granules, no differences are observed between the cell grown on agar and that grown in broth. The spirilla begin to move as soon as they are transferred into the liquid media.

Stained cell: The spirillum which is grown in the nutrient broth and the spirillum which is obtained from the colony on agar exhibit almost the same appearances. When the cell of 24 hrs. old is stained with Löffler's methylene blue, it shows somewhat different appearances, such as (1) the feature which is uniformly colored heavy blue without showing any structure, (2) the feature of several small volutin granules, taking purplish-red color, scattered in the body which is uniformly dyed blue or which reveals the so-called reticulate appearance. The volutin granules are most abundantly found in the cell after incubation for 2 days. The flagella are readily stained by Löffler's method. The young cells of the spirillum possess single flagellum or a tuft of 2 to 3 flagella at both poles of the body. The number of flagella increases as the culture becomes older. After 2 days or more, the cell possesses a tuft of several flagella at each pole. The spirillum is Gram-negative.

2. Cultural characteristics

Agar colonies: Growth is speedy. Colonies are seen with the naked eye after 24 hrs. After 48 hrs., surface colonies are circular or slightly irregular (1.0 to 1.4 mm. in diameter) with smooth surface and small indented margin, and are finely granular, convex, creamy-white, opalescent, and brittle. Deep colonies are smaller and round, elliptical, spindle or irregular.

Gelatine colonies: Colonies are seen with the naked eye after 48 hrs. After 5 days, surface colonies are punctiform or circular (0.9 to 1.1 mm. in diameter) with rough surface, and are lacerate, coarsely granular, creamy-white, opaque, and brittle. Gelatine plate is liquefied, forming a slightly concave surface. Deep colonies are the same as the surface colonies except that they are smaller, and that they have a small indented margin.

Agar stroke: Growth is moderate, beaded, creamy-white, glistening, and brittle. Fetid odor is absent, and the medium is unchanged.

Agar stab: Growth is seen along the entire stab line and on the surface, but scant in the lower part of the stab. The line of puncture is papillate, and the medium is unchanged.

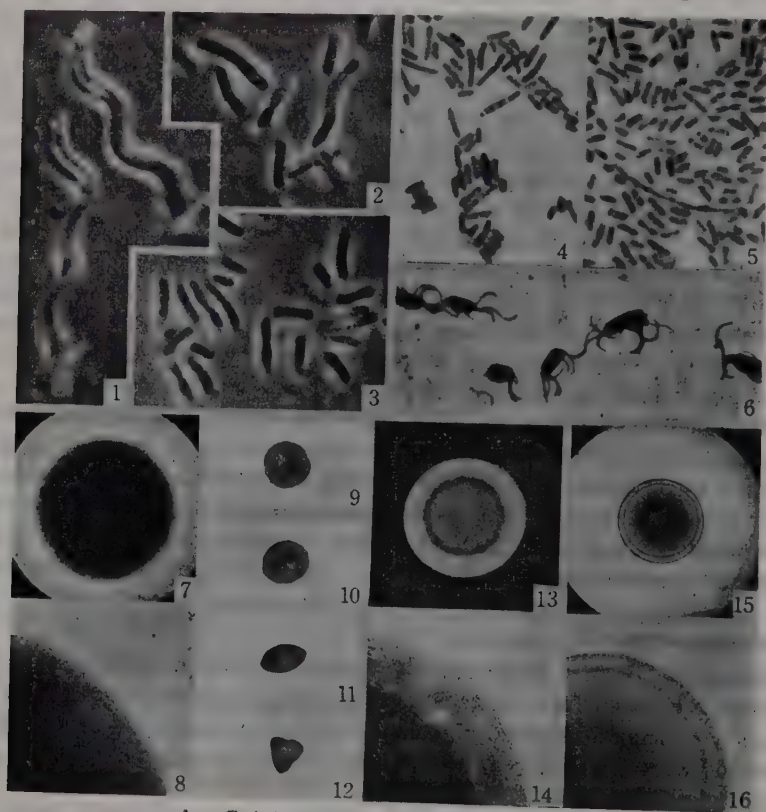
Gelatine stab: Growth is seen along the entire stab line, but scant in the lower part of the line. Liquefaction begins in 5 days. Form of liquefaction is napiform for about 10 days, but becomes infundibular as the culture becomes older. After 4

weeks, liquefaction is about 25 mm. in depth.

Nutrient broth: Growth is moderate. After 24 hrs., thin membraneous masses develop on the surface of medium along the tube wall. The masses precipitate by slight agitation. A thin ring remains along the tube wall after the masses have precipitated, and it disappears in about 5 days and finally the medium becomes clear. Fetid odor is absent.

3. Physiological characteristic

Growth appears on the surface and in upper ca. 3 mm. of an agar shake cultured,



A. *Spirillum metamorphum* nov. sp.

Figs. 1-16. Fig. 1, vegetative cells in initial culture, showing the definite spiral form. $\times 1200$. Fig. 2, vegetative cells in the nutrient broth after 24 hrs. at 30° , showing the vibrio- or the S-shaped form. $\times 1200$. Fig. 3, vegetative cells obtained from a colony on the nutrient agar plate after 24 hrs. at 30° , showing the short rod- or the vibrio-shaped form. $\times 1200$. Figs. 4 and 5, specimens stained with Löffler's methylen blue. $\times 1200$. The cells in Fig. 4 are obtained from a colony on the nutrient agar plate after 24 hrs. at 30° . The cells in Fig. 5 after 48 hrs. at 30° , containing the small volutin granules. Fig. 6, flagella-staining by Löffler's method. The cells are obtained from a colony on the nutrient agar plate after 24 hrs. at 30° . $\times 1200$. Fig. 7, a surface colony on the nutrient agar plate after 48 hrs. at 30° . $\times 15$. Fig. 8, the margin of the same colony as in Fig. 7. $\times 50$. Figs. 9 to 12, deep colonies on the nutrient agar plate after 48 hrs. at 20° . $\times 15$. Fig. 13, a surface colony on the nutrient gelatine plate after 5 days at 20° . $\times 15$. Fig. 14, the margin of the same colony as in Fig. 13. $\times 50$. Fig. 15, a deep colony in the nutrient gelatine plate after 5 days at 20° . $\times 15$. Fig. 16, the margin of the same colony as in Fig. 15. $\times 50$.

but not in the lower part. The spirillum does not reduce nitrate to nitrite, and produces neither indole nor hydrogen sulfide. Catalase is not produced by growth on the nutrient agar. The spirillum produces neither acid nor gas from glucose, fructose, lactose, sucrose and mannitol. Litmus milk is not coagulated, and shows a slight alkaline-reaction in ca. 1 week, but it is decolorized at bottom. This appearance does not change later. The optimum temperature for growth is 30°. The spirillum can grow at 37°, but not above 40°. The optimum pH is 7.6 to 8.4, and growth can be initiated between pH 6.0 and 9.6. Methyl red and Voges-Proskauer reaction are negative. The spirillum does not grow in synthetic media containing any of succinate, fumarate, malate, lactate, acetate, propionate, butyrate, citrate, malonate, glucose, fructose, glycerol and ethyl alcohol as a sole carbon source, and any of ammonium salts, nitrate, urea and asparagine as a sole nitrogen source.

Spirillum crassum nov. sp.

1. Morphological characteristics

Vegetative cell: The spirillum grown in the nutrient broth after incubation for 24 hrs. has a stout cell, with obtuse ends. It is generally 10 to 40 microns in length, but occasionally the cell reaches the length of 100 microns. The diameter of the cell is 1.3 to 1.5 microns. The waves are shallow, regular and 1 to 6 in number. The wave length is 6 to 7 microns. The width of the wave 1.3 to 1.5 microns. Refractive granules are present in some individuals but not in others. The spirillum moves to either direction with equal rapidity and facility, rotating counterclockwise. In old cultures, the vegetative cells predominate and the microcysts are not found. The cell taken from a colony on the nutrient agar plate of 24 hrs. old is shorter than that taken from the nutrient broth. A great number of the cells show the vibrio- or the short spiral-shaped form. The cell is generally 3 to 15 microns in length, but occasionally reaches the length of 20 microns or more. The diameter of the cell is almost the same as that of the cell grown in the nutrient broth. Concerning the refractive granules, no differences are observed between the cells grown on agar and that grown in broth. The spirilla begin to move as soon as they are transferred into the liquid media.

Stained cell: The spirillum cultivated in the nutrient broth and the spirillum taken from the colony on agar plate exhibit almost the same staining features. When the spirillum incubated for 24 hrs. is stained with Löffler's methylene blue, it shows the cell which is uniformly colored light or heavy blue, including several volutin granules. The reticulate structure is not clear. The volutin granules are most abundantly found in the cell of one to two days old cultures. The flagella are readily stained by Löffler's method. Most of the spirillum after 24 hrs. have a tuft of several to about ten or more flagella at both poles of the cell. The spirillum grown in the nutrient broth has as many flagella as the spirillum obtained from a colony on agar. The spirillum is Gram-negative.

2. Cultural characteristics

Agar colonies: Growth is speedy. After incubation for 24 hrs., surface colonies are circular or slightly irregular (1.0 to 1.5 mm. in diameter) with smooth surface, and are coarsely granular, convex, light brown, opalescent, and brittle. The outer part of the colony appears as a thin transparent layer, and the inner part is granular and has lacerate margin. The outer layer disappears after ca. 1 day. Deep colonies

are smaller than the surface ones, and are round, elliptical or irregular. Their margin are lacerate or lobate. As the culture becomes older, the surface colonies become larger and show a change in margin and in elevation. Namely, after 5 days the diameter of the colonies is 4.0 to 5.0 mm., the margin is undulate, and the elevation is umbonate. Deep colonies show the same characteristics as after 24 hrs. except that the diameter is 1.5 to 2.0 mm. and that the margin is lacerate in all colonies.

Gelatine colonies: Colonies are seen with the naked eye after 2 days. After 5 days, surface colonies are circular (1.0 to 1.6 mm. in diameter) with rough surface, and are coarsely opaque, flatt, brown, opaque, and brittle. Concerning the margin, there are seen the following three different types: the first is curled, the second lacerate, and the third ciliate. Deep colonies are punctiform (ca. 0.4 mm. in diameter), and have the lacerate margin. As the culture becomes older, the margin of the curled margin-colonies changes into lacerate or ciliate. After 1 week the surface colonies have the lacerate or ciliate margin, and show a concentric stratification with a filamentous structure. Gelatine plate is liquefied, forming a slightly concave surface. Deep colonies are filamentous (0.8 to 1.2 mm. in diameter), and brown.

Agar stroke: Growth is beaded, creamy-white, glistening, and brittle. Fetid odor is absent, and the medium is unchanged.

Agar stab: Growth is seen along the entire stab line, and on the surface. The line of puncture is papillate in ca. 1 week, but afterwards changes to beaded. The medium is unchanged.

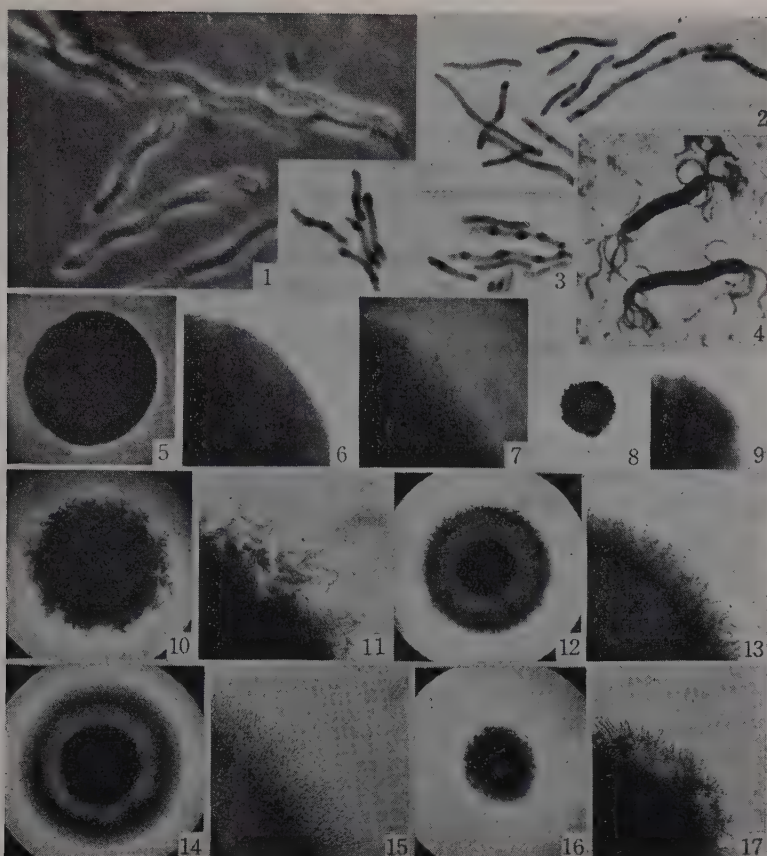
Gelatine stab: Growth is seen along the entire stab, but is scanty in the lower part. Growth on the surface develops fairly around the point of puncture. The line of puncture is beaded, and the gelatine is slowly liquefied in crateriform. After 4 weeks, liquefaction is ca. 8 mm. in depth.

Nutrient broth: Growth is speedy. After 24 hrs., a thin membrane develops on the surface. The medium is densely turbid. The membrane sinks into the bottom in a lump by slight agitation. After the membrane has sunk, the ring remains along the tube wall. The turbidity of the medium becomes light as the culture becomes older, but it still remains 2 weeks later. The ring still remains at that time. Fetid odor is absent.

Potato: No growth occurs.

3. Physiological characteristics

The spirillum grows on the surface and in upper ca. 3 mm. of an agar shake culture. It does not reduce nitrate, and does not produce indole and hydrogen sulfide. Catalase is not produced by growth on the nutrient agar. The spirillum produces neither acid nor gas from glucose, fructose, sucrose, lactose and mannitol. Litmus milk is unchanged. The optimum temperature for growth is 38 to 40°. The organism can grow at 45°, but not at above 48°. The spirillum grows well from the pH 6.5 to 8.5, and growth can be initiated between pH 5.8 and 9.4. Methyl red and Voges-Proskauer reaction are negative. The spirillum utilizes asparagine well and urea slightly as sole nitrogen sources in synthetic media, but not the salts of ammonium and nitrate. The spirillum utilizes the salts of succinic, fumaric, pyruvic, malic, and lactic acid, glucose and glycerol as sole carbon sources in synthetic media, but not the salts of acetic, propionic, butyric, citric, and malonic acid, fructose and ethyl alcohol.



B. *Spirillum crassum* nov. sp.

Figs. 1-17. Fig. vegetative cells moving slowly in the nutrient broth after 24 hrs. at 39°, photographed with a dark contrast phase microscope. $\times 1200$. Figs. 2 and 3, specimens stained with Löffler's methylen blue. $\times 1200$. The cells in Fig. 2 are obtained from a colony grown on the nutrient agar plate after 24 hrs. at 30°. The cells in Fig. 3 after 72 hrs. Fig. 4, flagella-staining by Löffler's methods. The cells are obtained from a colony grown on the nutrient agar plate after 24 hrs. at 39°. $\times 1200$. Fig. 5, a surface colony on the nutrient agar plate after 24 hrs. at 39°. $\times 15$. Fig. 6, the margin of the same colony as in Fig. 5. $\times 50$. Fig. 7, the margin of the colony after 5 days. $\times 15$. Fig. 8, a deep colony on the nutrient agar plate after 24 hrs. at 39°. $\times 15$. Fig. 9, the margin of the same colony as Fig. 8. $\times 50$. Figs. 10, 12 and 14, surface colonies on the nutrient gelatine plate after 5 days at 20°. $\times 15$. Figs. 11, 13 and 15, each margin of the same colonies as in Figs. 10, 12 and 14. $\times 50$. Fig. 16, a deep colony on the nutrient gelatine plate after 5 days at 20°. $\times 15$. Fig. 17, the margin of the same colony as in Fig. 16. $\times 50$.

Discussion

Spirillum metamorphum is thicker than 1 micron in the diameter of the cell. Although the initial culture of the spirillum contains the definite spiral cells in high percentage, the subcultures scarcely contain the definite spiral forms. Even when the spirillum shows the spiral form, it is nothing but a chain of cells which is caused

by successive cell division. As *Spirillum* which possesses such morphological characteristics, the following two species have been reported: *Spirillum kutscheri* Migula^{2,3,4}) and *Spirillum giesbergeri* Williams et Rittenberg⁵). The spirillum isolated by the present author can not be distinguished morphologically from those species because it is markedly similar to them also in the other morphological properties. The spirillum in question, however, differs from them in some cultural and physiological characteristics. The differences among these spirilla are expressed in Table 1.

Table 1. The differences among *Spirillum kutscheri*, *Spirillum giesbergeri*, and *Spirillum metamorphum* nov. sp. isolated by the present author.

	<i>Spirillum kutscheri</i> Migula	<i>Spirillum giesbergeri</i> Williams et Rittenberg	<i>Spirillum metamorphum</i> nov. sp.
Agar colony	deep; dark brown; round or whetstone-form	surface: white; lobate	surface: creamy-white; small indented margin
Agar stab	filiform		deep: creamy-white; round, elliptical or spindle
Gelatine colony	surface: transparent; whetstone-form or round; lobate		papillate
Gelatine stab	slow liquefaction*	no liquefaction	surface: creamy-white; punctiform or circular; lacerate
Potato	limited growth*	very slight, white growth	liquefaction; napiform in 10 days, but infundi- bular afterwards
Catalase	positive*	positive	no growth
Optimum temperature	22-27°		negative
Available nitrogen compound	ammonium salts*	ammonium salts, nitrate, urea, and asparagine	30-32°
Available carbon compound	salts of malic, and pyruvic acid*	salts of pyruvic, malic, and lactic acid	no growth in synthetic media
	salts of malic, and succinic acid**		

* Descriptions according to Giesberger's monograph⁶).

** Descriptions according to Bergy's Manual.

As shown in Table 1, the spirillum isolated by the present author differs from *Spirillum kutscheri* and *Spirillum giesbergeri* in eight important respects for the identification. The most striking characteristics by which the spirillum in question is distinguished from the above mentioned two species are its inability to grow on potato, the absence of catalase, and its inability to grow in synthetic media. From the above-mentioned evidence, the spirillum isolated from *Corbicula japonica* Prime is diagnosed as a new species, and it is named *Spirillum metamorphum* from its metamorphosis.

Spirillum crassum isolated from *Cipangopaludina malleata* (Reeve) distinctly differs from all definite species^{4,5,6}) described up to the present time in several important characteristics. Especially the following characteristics are sufficient to diagnose the spirillum as a new species. Namely, (1) the spirillum has a stout cell of 1.3 to 1.5

microns in diameter; (2) the optimum temperature for growth is 38 to 40°, at which most of species can not grow; (3) the spirillum is catalase-negative; (4) the spirillum can utilize asparagine well, but urea is less readily utilized; the salts of ammonium and nitrate are not utilized as a sole source of nitrogen for growth; (5) the spirillum can utilize the salts of succinic, fumaric, pyruvic, lactic, and malic acid, glucose and glycerol as sole carbon sources, but not utilize the salts of acetic, propionic, butyric, citric, and malonic acid, fructose and ethyl alcohol. From the above points of view, the spirillum isolated from *Cipangopaludina malleata* (Reeve) is diagnosed as a new species, and it is named *Spirillum crassum* because it has a stout cell.

Summary

Two new species of *Spirillum* have been isolated from the putrid media in which the putrid bodies of two species of the fresh water shell fishes, *Corbicula japonica* Prime and *Cipangopaludina malleata* (Reeve) laid. On the basis of the morphological, cultural and physiological characteristics, the spirillum isolated from *Corbicula japonica* Prime is diagnosed as a new species, and it is named *Spirillum metamorphum* from its metamorphosis; and also the spirillum isolated from *Cipangopaludina malleata* (Reeve) is diagnosed as a new species and named *Spirillum crassum* because it has a stout cell.

The author is grateful to Dr. Teijiro Kishitani, the president of Suzugamine Women's College, for his valuable advice and encouragement during the course of the study, and thanks are due also to Mr. Morio Katakawa, the chief of Medical branch of the library attached to Hiroshima University, for his kindness in collecting literature which has been cited.

References

- 1) Terasaki, Y., Bot. Mag. Tokyo, 74: 79 (1961).
- 2) Kutscher, Cent. f. Bakt., I Abt., 18: 614 (1895).
- 3) Migula, W., System der Bakterien Bd. II: 1024, Jena (1900).
- 4) Breed, R. S., Murray, E. G. D., and Smith, N. R., Bergy's Manual of Determinative Bacteriology 7th ed., Baltimor (1957).
- 5) Williams, M. A., and Rittenberg, S. C., International Bull. of Bacteriological Nomenclature and Taxonomy 7: 49 (1957).
- 6) Giesberger, G., Beiträge zur Kenntnis der Gattung *Spirillum* Ehb., Delft (1936).

摘 要

寺崎弥助: *Spirillum* 二新種について

1958年10月、広島市鷹の橋マーケットで購入したヤマトシジミ、および、1959年5月、広島郊外五日市町海老塩浜の稲田で採集したマルタニシの腐敗液から、寒天平板塗抹法により *Spirillum* をそれぞれ一種ずつ分離した。分離に用いた培地成分は、前者では、ペプトン、酵母エキス、ヤマトシジミせんじ汁、食塩であり、後者では、肉エキス、ペプトン、酵母エキスである。両者を培養し、形態学的、培養的、生理学的性質をしらべ、正確な種として記載された *Spirillum* と比較検討した結果、それらを新種と断定した。ヤマトシジミより分離した *Spirillum* は、分離当初においては明瞭ならせん形を示したが、継代培養数回後には体長の短い棒状あるいは *Vibrio* 状に変形し、現在も同様な形態を示している。まれにらせん形の個体がみられるが、二個あるいはそれ以上にくびれている場合が多い。この形態変化により、この菌を *Spirillum metamorphum* と命名した。マルタニシより分離した *Spirillum* は菌体が太く強固であることにより、*Spirillum crassum* と命名した。(鈴峰女子短期大学生物学教室)

Chlorophyll Content in Sessile Algal Community of Japanese Mountain River

by Hiromu KOBAYASI*

Received November 8, 1960

In the last decade, the attention of limnologists has been focused on the production of dry matter in lakes and the intensive studies have been carried out by many investigators. While the similar investigation on the running waters has not as yet been performed adequately except for several researches made by Odum^{1,2}), McConnel and Sigler³).

In a mountain river, sessile algae are important primary producers of organic matter and the main feed for sweetfish, therefore the information on the dry matter production of sessile algal community is indispensable as a background for the study of the ecosystem in a mountain river. However, the knowledge in this field has lagged also in Japan, though a few of the taxonomic and floristic studies have already been reported.

To fill the foregoing gaps, the author has undertaken a series of ecological studies of sessile algae in the mountain section of the River Arakawa during 1959. In this paper, he will describe the standing crop of algal community measured in chlorophyll amount and then discuss the habitat factors affecting the production of algae.

Main features of the River Arakawa

Arakawa is arising from a portion of the Titibu Mountains in Saitama Prefecture, Central Japan and terminating in Tokyo Bay. As seen in Figs. 1, 2, the watercourse is divided into the mountain river and the downstream region. The mountain river is a clear, rapid stream and it can be furthermore divided into two parts; the canyon section and the lower section. The former is 0.2 to 0.5 m. in depth, 5 to 15 m. in width and has a steep gradient. The latter has a gradual slope, a depth of 0.2 to 0.7 m. and a width of 10 to 30 m. As pointed out by Berg⁴), it seems ecologically reasonable to divide the mountain section into the said parts. The river bed of the mountain section is occupied by rocks of about 10 to 20 cm. in diameter.

The downstream region from Kumagaya to the estuary is characterized by high turbidity and a heavy silt deposition.

Methods

In the present study 20 stations were selected in 95 km. length of the mountain river section from Akasawa to Kumagaya. For the measurement of chlorophyll content and cell number of sessile algal community, 4–5 rocks were collected at random from the river bed of each station. The algae on each rock surface of 5×5 cm.² were left from brushing away by protection of a sheet of the same area of polyethylene cloth. The protected algae of these rocks were collected into a bottle and

* Botanical Institute, Faculty of Science, Tokyo University of Education, Otsuka, Tokyo, Japan.

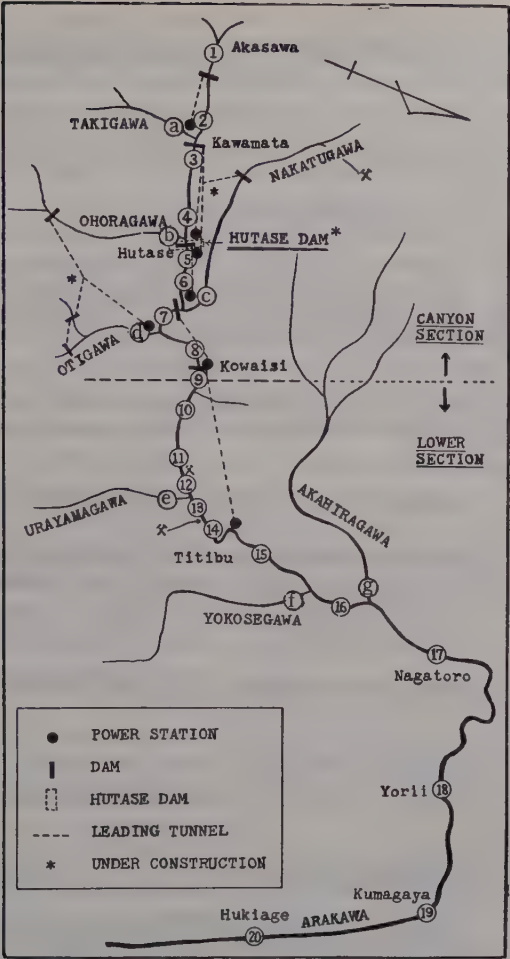


Fig. 1. A map showing the watercourse of the River Arakawa in the Titibu Mountain district.

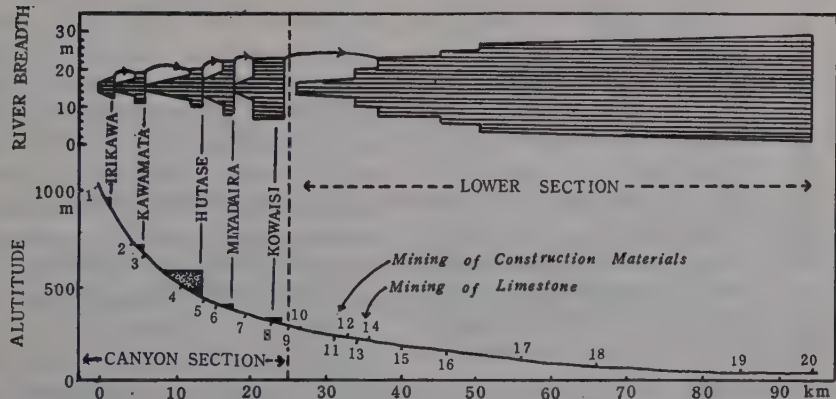


Fig. 2. Profile of the River Arakawa in the mountain district, showing gradients, sampling stations and river breadths.

Table 1. Chlorophyll amount and cell numbers in the sessile algal community of the mountain section of the River Arakawa.

Station	January-March				April			July-August			November			Average
	Date Time	W.T.	Chl. γ /cm. ²	Cell $\times 10^4$ /cm. ²	Date Time	W.T.	Chl. γ /cm. ²	Date Time	W.T.	Chl. γ /cm. ²	Date Time	W.T.	Chl. γ /cm. ²	
1	{ 13.45 16.00 9.00 Jan. 2	0	11.9	234	{ 13.00 15.30 16.00 Apr. 4	5	2.5	{ 12.00 13.30 9.20 July 5	13	5.4	{ 14.00 11.24 10.49 10.35 16.36 16.43 Nov. 17	5	8.7	7.1
2		0	1.5	115		8	1.0		14	1.2		6	4.7	2.1
a		2	1.9	31		6	0.6		14	2.1		6	1.8	1.6
3	{ 10.00 10.30 11.00 Jan. 1	2	0	0	{ 16.40 17.20 17.40 July 5	6	1.7	{ 10.30 13.00 13.15 15.40 15.50 16.05 16.30 16.40 17.00 July 7	14	1.2	{ 10.35 16.36 16.43 Nov. 17	6	2.1	1.2
4		1	1.3	21		8	1.3		18	1.2		8	7.4	2.8
b		1	1.3	25		8	1.0		18	1.2		8	18.3	5.4
5	{ 11.30 11.00 12.00 Feb. 1	2	1.5	42	{ 10.00 10.20 10.30 Apr. 5	7	0.6	{ 13.00 13.15 15.40 15.50 16.05 16.30 16.40 17.00 July 5	17	0.2	{ 16.43 16.00 15.25 15.35 14.53 14.14 13.34 Nov. 17	7	3.6	1.4
6		3	7.2	117		7	0.9		17	0.7		8	10.8	4.8
c		3	0.6	38		9	0.9		18	0.3		8	10.8	3.1
7	{ 12.00 12.20 13.00 Feb. 1	3	0.8	10	{ 10.30 11.00 11.20 Apr. 5	9	1.0	{ 15.40 16.30 16.40 17.00 July 5	21	0.3	{ 15.25 15.35 14.53 14.14 13.34 Nov. 17	9	1.4	1.0
d		1	3.2	95		9	9.9		19	0.7		9	41.4	13.8
8		4	0.5	5		10	0.7		19	1.8		10	6.6	2.4
9	{ 14.00 11.0 11.25 Jan. 25	4	—	—	{ 12.20 12.30 12.45 Apr. 5	10	2.7	{ 17.00 9.30 10.0 July 7	19	—	{ 13.34 12.35 11.50 11.30 11.25 10.55 10.25 9.38 16.45 15.15 15.20 14.42 13.14 12.05 10.20 Nov. 15	—	—	—
10		6	0.2	9		11	2.4		19	5.6		12	1.5	2.5
11		7	1.3	18		11	1.0		20	3.3		12	8.6	3.9
12	{ 11.25 13.30 14.00 Jan. 25	5	1.3	34	{ 13.00 13.30 13.50 July 7	11	0.3	{ 10.15 10.20 10.45 11.40 12.35 9.00 8.30 8.20 Aug. 7	20	1.3	{ 11.30 11.25 10.55 10.25 9.38 16.45 15.15 15.20 14.42 13.14 12.05 10.20 Nov. 15	12	1.7	1.3
e		4	0.3	19		11	0.3		20	4.0		9	10.5	3.8
13		6	1.6	95		11	0.3		20	4.9		11	2.2	2.3
14	{ 13.40 15.00 8.00 Mar. 2	6	0	0	{ 14.10 15.00 10.00 Apr. 6	11	1.7	{ 10.45 11.40 12.35 9.00 8.30 8.20 Aug. 7	22	6.0	{ 11.25 10.55 9.38 16.45 15.15 15.20 14.42 13.14 12.05 10.20 Nov. 15	11	10.5	4.6
15		5	0	63		11	1.1		22	3.1		8	13.8	5.0
f		5	2.0	8		12	0.9		21	1.3		12	11.2	3.5
16	{ 9.00 11.30 12.54 Mar. 2	8	0.5	18	{ 10.00 10.35 11.50 Apr. 6	12	1.5	{ 12.35 9.00 8.30 8.20 Aug. 7	21	4.6	{ 10.25 11.25 12.40 Nov. 15	10	10.7	3.6
g		6	1.5	3		12	1.5		21	2.1		10	10.7	4.8
17		9	0.8	39		13	2.8		21	2.1		12	10.7	3.6
18	{ 15.00 14.00 14.30 Mar. 2	8	1.0	17	{ 13.00 12.00 14.30 Apr. 6	13	2.8	{ 9.10 10.25 11.25 12.40 Nov. 15	24	14.0	{ 15.20 14.42 13.14 12.05 10.20 Nov. 15	11	17.0	7.2
19		8	0.5	8		12	0.6		25	11.0		11	17.0	7.3
20		12	0.4	29		14	0.9		28	13.3		13	28.3	11.2

homogenized with an ordinary home mixer. The homogenized sample was divided into two parts. One part was stored in a plastic bottle after fixed with 3% formalin solution for the count of cell number which was made by the Sedywick method. The other filtrated with a filter paper (Toyo No. 5a) for chlorophyll measurement after Hogetsu and Ichimura⁵).

Results and Discussion

1. Locational and seasonal changes in chlorophyll content in the mountain river

The measurement was carried out four times in a year. The results are summarized in Table 1, in which those obtained in the tributaries are also included. During January to March 1959, the river bed at Station 3 and 9 was dried up, therefore the sampling for chlorophyll measurement could not be performed. At Station 14 the river bed was densely covered with white deposit of fine lime stone outflowed from a settling pool of Titibu Industry. Thereby the chlorophyll content in the region below Station 14 was remarkably reduced. Reduction of the standing crop of algae referred to the precipitation of the coarse bed load was also observed at Station 12 in a series of measurements made in summer and fall.

Fig. 3-A shows the annual mean value of chlorophyll amount per unit surface of the river bed deduced from the data in Table 1. In general, it can be said that the chlorophyll amount is larger in the lower section than in the canyon section. The mean value of 0.003 mg./cm.^2 and 0.007 mg./cm.^2 was respectively obtained in the area of canyon section and in the lower section. Furthermore, it is interesting to note that a regular rhythm can be seen in the locational change of chlorophyll amount within a mountain section. The low points in the rhythm were generally found at the station departed a short distance downwards from the hydroelectric impoundment. This fact may suggest that there is a close correlation between the variation of the standing crop of algal community and the abrupt change in water level induced by the artificial impoundment. Namely, a great part of the water mass discharged from a hydroelectric impoundment is usually sent through underground tunnel to the lower station situated just above the next impoundment, thereby the water level

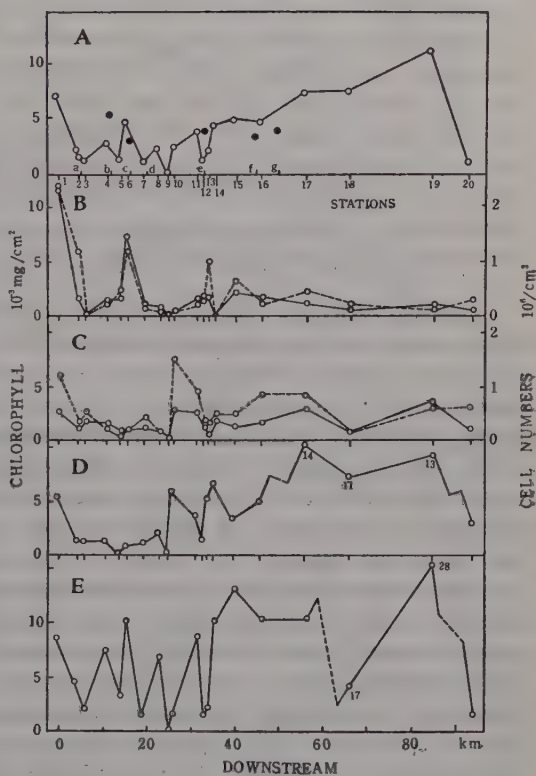


Fig. 3. Locational changes in chlorophyll content and cell number in the sessile algal communities of the mountain section of the River Arakawa. (A) Average, (B) January to March, (C) Spring, (D) Summer, (E) Fall. —○— Chlorophyll amount ---○--- cell number, ● chlorophyll in tributaries.

in the river remarkably fluctuates at downstream region of the impoundment and consequently the aquatic environment of the river bed is also extremely unstable. On the other hand, the aquatic environment of the lower section of the mountain river on the whole indicates pretty stable state. As indicated in Fig. 4, the locational difference in chemical components of the river water could not be found definitely, therefore the poor chlorophyll content in the canyon section may partly be attributed to the foregoing changeable aquatic environment.

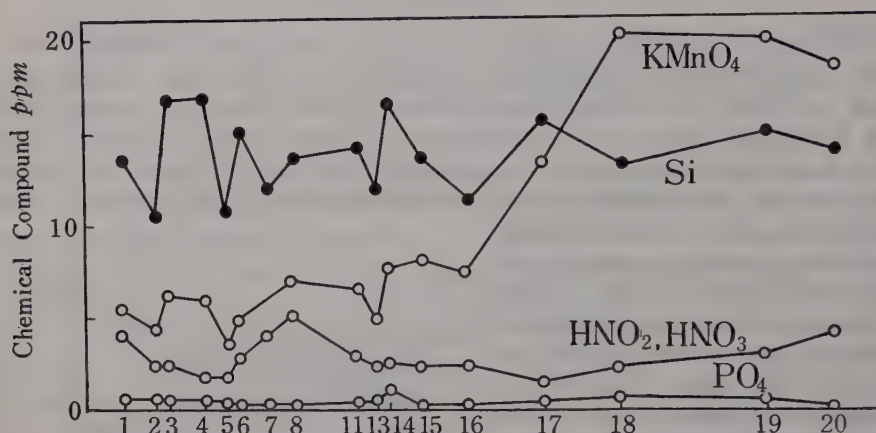


Fig. 4. Locational changes in dissolved solids. PO_4 , HNO_2 , HNO_3 ; $\times 10^{-1}$. The data were obtained by Hayakawa during period of Dec. 1958 to Jan. 1959.

Definite seasonal change in the chlorophyll content was observed within the entire region of the mountain section but the pattern of the chlorophyll change in the canyon section differed a little from that in the lower section. As can be seen in Fig. 3, B-E, the chlorophyll content in the lower section began to increase in spring, reached a maximum in fall and reduced in winter. Therefore, such a feature of the seasonal change resembles that in lakes, because Ichimura⁶⁾ has observed in the annual rhythm of chlorophyll content of lake water, one or two pulses, the peak of which occurs usually in spring and fall. On the contrary, in the canyon section the peak was obtained in winter. Presumably rich chlorophyll content in winter may be attributed to the characteristic life of *Hydrurus foetidus*, which is dominant species in the mountain river, and especially the winter vegetative thalli which develop about 10 cm. in length cover densely the river bed of the upstream portion of the canyon section. As can be seen in Table 1, the chlorophyll content in the tributaries was roughly the same as that obtained in the main river near the outlet of each tributary. As an exceptional case, however, a large quantity of chlorophyll was measured at Station d in the River Otigawa. It was 99 mg./m.² in April and 414 mg./m.² in November. Comparing the chemical character of the River Otigawa with those of the other streams, no reliable reason was found to expect such an enormous algal production. The algal flora of the River Otigawa is characterized by *Prasiola japonica*, which is abundantly observed only in this river within the Titibu Mountain district.

2. Comparison of chlorophyll amount in the River Arakawa with that in other fields

According to the theoretical elucidation made by Steemann Nielsen⁷⁾, the absolute maximum chlorophyll content in lake is 300 mg./m.² in the photic layer from the

surface down to the depth where the light is reduced to one per cent. The chlorophyll amount contained in the trophogenic layer is about 30–80 mg./m.² in ordinary eutrophic lakes and 100–200 mg./m.² in extremely eutrophic lakes in Japan. Manning and Juday⁸⁾ measured 15–65 mg./m.² of chlorophyll in five Wisconsin lakes. According to the data presented by Gessner⁹⁾, the chlorophyll amount found in eutrophic lake, Wessling See was rather extraordinarily high (606 mg./m.²) but it was 148 mg./m.² in the photic layer. Therefore, the amount of chlorophyll in the mountain section of the River Arakawa can be comparable to that in ordinary eutrophic lakes but is smaller than those in the extremely eutrophic lakes.

According to McConnel and Sigler³⁾, the average chlorophyll content in the canyon section of the Logan River, Utah, is about 300 mg./m.², while that in the canyon section of the River Arakawa is only 25 mg./m.². Besides the injurious effect of the impoundments, this low value may be referred to the geographical difference in the habitat conditions. Especially, the River Arakawa frequently rises in case of heavy rain and typhoon, through which the river bed is disturbed and the benthic algal communities are destroyed by such physical action as scarping, burying etc.

The assessment of standing crop of phytoplankton community based on its chlorophyll content now becomes usual. The data obtained in this study show the fact that the proportion of chlorophyll to dry matter of algae is 0.6–0.8% in diatom, 0.8–1.2% in blue-green and 1.0–2.0% in green algae. Assuming 1.0% chlorophyll as the mean value in the algal community of the River Arakawa, the standing crop of sessile algae is 7.0 g./m.² as dry matter in the lower section and 2.5 g./m.² in the canyon section. Such values may somewhat ambiguous because of the specific and seasonal variations in the proportion of chlorophyll content to organic matter of algae.

3. Relation between the amount of chlorophyll and cell number in the sessile algal community.

Fig. 5, founded on the data taken from various stations along the watercourse, depicts a rough linear correlation between chlorophyll content and cell number. From the above result, it can be summarized that the locational floristic diversity in the sessile algal community is not striking in the mountain region of the River Arakawa.

As the result of the research on the algal flora of the River Arakawa, the dominant species in the canyon section were *Hydrurus* and *Ulothrix* in winter, *Achnanthes*, *Diatoma*, *Synedra* and *Ceratoneis* in spring and fall, and *Phormidium* and *Chamaesiphon* in summer. In the lower section, they were *Hydrurus* in winter, *Achnanthes*, *Synedra* and *Ceratoneis* in spring and fall, and *Phormidium*, *Chamaesiphon* and *Cladophora* in summer. Therefore, the slight fluctuation in the linear correlation between chlorophyll amount and cell number may be referred to the local seasonal deviations in the floristic constitution of the algal community. In some stations, however, the changes in the chlorophyll content and cell number were not parallel. In the samples taken during January to March and in April, the mean value of chlorophyll amount per cell was about 400×10^{-11} mg.,

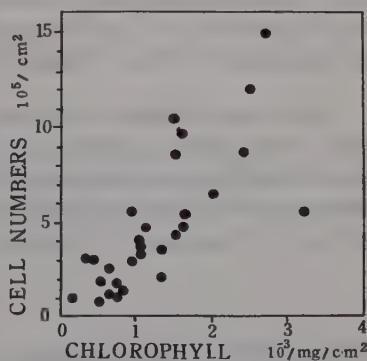


Fig. 5. Relation between chlorophyll and cell number in the sessile algal communities of the River Arakawa.

but the range of fluctuation was from 128×10^{-11} to 1056×10^{-11} mg. Presumably such fluctuation may be speculated from the difference of cell volume peculiar to species. Namely, the low value of chlorophyll amount per cell was attributed to the algal community which consisted of a great number of minute-sized algae, and high value was characterized by a small number of large-sized algae. For these reasons, the cell number, which has hitherto been employed as the measure of the standing crop of algae, may have little meaning from the ecological view-point. In determination of the quantity of algae, the chlorophyll method seems to be more reasonable than the counting one, and this has already been proved by many investigators who used the chlorophyll content as an index of productivity of waters.

Summary

As a first step to study the primary production in the mountain section of the River Arakawa, the standing crop of the sessile algal community was measured by means of the chlorophyll method. From the ecological point of view, the mountain section was divided into two parts; the canyon section and the lower section.

1. The annual mean value of chlorophyll content on the river bed was 25 mg./m.² in the canyon section and 70 mg./m.² in the lower section. These values coincide fairly well with those measured in the ordinary eutrophic lakes in Japan.

2. Throughout the watercourse, definite rhythm was observed in the seasonal changes of chlorophyll content on the river bed. The peaks were found in winter in the canyon section, while those in lower section were in fall.

3. There is a linear correlation between the chlorophyll content and cell number in the sessile algal community of the mountain river.

4. The main factors determining the standing crop of the sessile algal community in the mountain section of the River Arakawa can be summarized as follows; (1) unstable aquatic environment of river bed caused by the hydroelectric impoundment, (2) physical action such as scraping and burying referred to the flood of river by heavy rain, and (3) the precipitation of the coarse bed load originated from the construction of the impoundment and of the fine lime stone outflowed from a settling pool of Titibu Mining Industry.

The author wishes to express his sincere thanks to Prof. H. Ito and Dr. S. Ichimura of the Tokyo University of Education for their instructive advice and suggestion throughout the progress of this study. Thanks are also due to the staffs of the Hutase-Dam-Kensetu-Suisankagaku-Tyosadan for their kind support and efforts, and to Mr. K. Miura and C. Asakawa for their kind assistance.

References

- 1) Odum, H. T., *Limnol. Oceanogr.*, **1**: 102 (1956).
- 2) —, *ibid.* **2**: 85 (1957).
- 3) McConnel, J. W., and Sigler, W. F., *ibid.* **3**: 335 (1959).
- 4) Berg, K., *Verh. Int. Ver. Theoret. Ang. Lim.* **10**: 76 (1949).
- 5) Hogetsu, K., and Ichimura, S., *Jap. Jour. Bot.* **14**: 280 (1954).
- 6) Ichimura, S., *Bot. Mag. Tokyo* **69**: 7 (1956).
- 7) Steemann Nielsen, E., *Physiol. Plantarum* **10**: 1009 (1957).
- 8) Manning, W. M., and Juday, R. E., *Trans. Wis. Acad. Sci., Arts and Lett.* **33**: 363 (1941).
- 9) Gessner, F., *Schw. Zeitschr. f. Hydrologie* **11**: 378 (1949).

摘 要

小林 弘： 溪流の底生藻類群落のクロロフィル量について

河川の底生藻類の定量のためには、一般に個体数計数法が用いられているが、筆者は湖沼や海洋で広く行なわれるようになったクロロフィル法の数々の長所に着目し、同法の河川への応用を試みた。1959年の調査によって得られた荒川流域の底生藻類の年平均クロロフィル量は上流部で、 25 mg./m.^2 、下流部で 70 mg./m.^2 で富栄養湖の単位表面積あたりのクロロフィル量に相当する値であった。河川の底生藻類の生育にとって、(1) ダム工事、同骨材採取、鉱石の採掘などのために作りだされた土砂やその他の粒状物質の河底の礫上への堆積、(2) 水力発電用小型ダムによって引き起こされる、その下流域の激しい水位の変化、(3) 降雨、台風などにともなう河川の増水、などがもっとも注目すべき要因として認められた。また、クロロフィル量の測定と平行して行なった個体数計数の結果との比較から、前者がより基礎生産の研究には合理的であることを論じた。(東京教育大学理学部植物学教室)

Studies on the Flower Initiation of Spring Wheat in Sterile Culture

I. Effects of Photoperiods and Photo-sensitive Growth Stage

by Kiyomi WADA

Received November 10, 1960

Recently many studies on flower initiation have been carried out, using the plants cultured aseptically in test tubes containing the artificial culture medium. It is known that many plants initiate flower primordia in total darkness^{1,2,4,5}), and even at cool temperature³). Sugino⁶) reported that the spring wheat, Konosu No. 25, initiated flower primordia under continuous illumination, 8-hour short days, and complete darkness.

The present experiments were performed with the aim to examine the effects of photoperiods upon flower initiation and growth of spring wheat cultured in test tubes.

Material and Methods

One of the most early-flowering varieties of spring wheat, Konosu No. 25, was used in the present experiments. This plant requires no chilling and has a very short life cycle and initiates visible ear primordia two weeks after germination under continuous illumination.

For seed sterilization, well-matured, and medium-sized seeds were selected. They were 1) washed with a germicidal soap, 2) sucked with vacuum pump in 70 per cent ethyl alcohol solution for 2 minutes, 3) immersed in 10 per cent solution of chlorinated lime for 30 minutes, and then 4) washed with distilled and sterilized water several times. The sterilized seeds were sown aseptically, one grain per test tube containing: the modified White's medium consisting of 200 mg. $\text{Ca}(\text{NO}_3)_2$, 360 mg. MgSO_4 , 200 mg. Na_2SO_4 , 80 mg. KNO_3 , 65 mg. KCl , 16.5 mg. KH_2PO_4 , 4.5 mg. MnSO_4 , 1.5 mg. ZnSO_4 , 1.5 mg. H_3BO_3 , 0.75 mg. KI , 2.5 mg. Fe-citrate , 40 g. sucrose, 8 g. agar and 1,000 ml. distilled water.

The light conditions were as follows: continuous illumination, 8-hour short days, and total darkness. The plants were grown under various combinations of these three light conditions. In the experiments started on October 7, 1955, and February 20, 1956, the source of artificial light consisted of four 100 watt incandescent lamps and one 20 watt daylight fluorescent lamp, and the luminosity at the plant level was about 1,000 lux. In the daytime, they were supplemented with diffused daylight which had about one fiftieth of natural daylight intensity. In the experiment started on January 10, 1958, only the artificial light was used, and the luminosity at the plant level was about 2,000–3,000 lux.

All experiments were performed in an air-conditioned room at $20 \pm 2^\circ$. In the experiments mentioned above, the observations were carried out 35–50 days after germination. And, in the experiment started on December 11, 1956, in which the plants were cultured only in total darkness, the observations were carried out 110–160 days after germination. The flowering stage and the number of leaves produced

* Biological Institute, Faculty of Liberal Arts and Science, Shizuoka University, Shizuoka, Japan.

on the main stem (leaf number) were observed under a dissecting microscope. The value of flowering stage was recorded according to the arbitrary scale shown in Table 1.

Table 1. Values assigned to stages in development of the flower primordia.

Values	Description of the shoot apex
0	Vegetative, smooth dome
0.5	Slightly elongated apex
1	Elongated phalloid apex
2	Double ridge
3	Formation of side spikelet
4	Anther initials visible
5	Heading

Table 2. Culture of Konosu No. 25 under various photoperiodic treatments, from October 7 to November 10, 1955.

L (The continuous illumination): 24-hour photoperiod+no dark period.

S (The short day): 8-hour photoperiod+16-hour dark period.

Photoperiodic treatment (days)	Flowering percentage	Position of flower, indicated by leaf number	Flowering stage	Height of plants (mm.)	Number of plants observed
35L + 0S	100	5.1	4.2	99	11
20L + 15S	100	5.2	4.1	93	15
10L + 25S	100	5.2	3.7	74	24
5L + 30S	100	6.0	3.2	68	19
10S + 25L	100	6.0	3.9	79	19
20S + 15L	100	6.5	2.8	38	13
35S + 0L	100	7.0	2.3	25	8

Results

Depression of flowering and growth under prolonged short day treatment and darkness: All plants, subjected to continuous illumination, 8-hour short photoperiod, or both light conditions with various combinations, initiated flower primordia. With increasing exposure to 8-hour short days, however, the values of flowering stages and the heights of plants decreased, and the leaf number, which indicated the position of the node from which the plant entered into reproductive phase, increased gradually (Table 2). With increasing duration of dark culture, the values of flowering stages and fresh- and dry-weights decreased. The leaf number increased with increasing duration of dark culture. Although the heights of plants increased to considerable extent in the prolonged darkness, this increase may be due to etiolation (Tables 3, 4). Thus flowering and growth were depressed under the prolonged short days, and especially in darkness.

Promoting effects of photoperiods inserted in continuous darkness on flowering and growth: In order to examine the photoperiodic sensitivity of the dark-grown plants at various growth stages, the plants were cultured in darkness, and exposed to 5 or 10 day continuous illumination 0, 5, 10, 20 days after the germination (Tables 3, 4).

With the light interception for 10 days, all plants initiated flower primordia, irrespective of the growth stage during which the light was given. When the light was given 10 or 20 days after germination, the values of flowering stages, fresh- and dry-weights of plants decreased, and the leaf number increased as compared with those exposed to the light at earlier growth stages. When the 5 day light interception was given at the beginning of the sixth day after germination, flowering percentages showed higher values (95 and 83%) as compared with those given to the other growth stages, and the values of flowering stages also increased (Tables 3, 4).

Thus the results show that 1) the photoperiods inserted in continuous darkness

Table 3. Culture of Konosu No. 25 under various photoperiodic treatments, from February 20 to April 11, 1956.

L: continuous illumination. D: continuous darkness.

Photoperiodic treatment (days)	Flower-ing percent-age	Position of flower, indicated by leaf number	Flower-ing stage	Height of plants (mm.)	Fresh weight of plants (mg.)	Dry weight of plants (mg.)	Number of plants observed
50L + 0D	100	5.1	5.0	116	362	51	15
20L + 30D	100	5.0	4.6	182	318	59	24
10L + 40D	100	5.4	2.5	112	250	50	22
5L + 45D	89	7.3	1.0	95	260	37	26
5D + 5L + 40D	95	6.7	1.6	90	228	39	22
10D + 5L + 35D	57	7.6	1.9	101	215	39	28
5D + 45L	100	5.4	4.2	112	314	39	19
10D + 40L	100	6.1	2.6	73	248	40	28
20D + 30L	100	7.2	1.5	148	251	35	18
50D + 0L	0	7.0*	0.0	182	193	28	27

* The number of leaves produced in vegetative growth.

Table 4. Culture of Konosu No. 25 under various photoperiodic treatments, from January 10 to March 3, 1958.

L: continuous illumination. D: continuous darkness.

Photoperiodic treatment (days)	Flower-ing percent-age	Position of flower, indicated by leaf number	Flower-ing stage	Height of plants (mm.)	Fresh weight of plants (mg.)	Dry weight of plants (mg.)	Number of plants observed
50L	100	5.0	5.0	101	447	139	25
50D	0	7.5*	0.0	216	171	37	30
5L + 45D	80	7.3	1.3	139	187	45	25
5D + 5L + 40D	83	7.5	1.8	176	231	52	35
10D + 5L + 35D	79	8.4	1.5	146	154	38	35
15D + 5L + 30D	75	8.5	1.4	155	167	42	35
20D + 5L + 25D	77	8.9	1.4	180	170	40	35
10D + 10L + 30D	100	6.7	3.2	172	199	56	35
20D + 10L + 20D	100	7.2	3.0	187	196	59	35

* The number of leaves produced in vegetative growth.

promote flowering and growth, 2) the light period of 5 days is not enough for flower promotion but that of 10 days is sufficient, and 3) the growth stage from the sixth day to the tenth day after germination is the most sensitive one to photoperiodic treatment.

Discussion

The study on the flowering of spring wheat, Konosu No. 25, cultured *in vitro* was published by Sugino⁶) in 1957. He reported that this plant initiated flower primordia under three light conditions (total darkness, 8-hour short days, and continuous illumination), and that sucrose, more than 4 per cent, must be added to the medium in dark culture, 2 per cent sucrose in short days culture, and no sucrose in continuous illumination. He concluded that "...Especially, in dark culture, the amount of sugar added to the medium has a very significant influence not only upon the survival of plants but also upon the flowering in wheat....".

In the present experiments, the photoperiods given to dark culture showed the promoting effect on flowering and growth of the plant. On the other hand, it was shown that flowering and growth were depressed with the prolonged short days and darkness. These depression may be due to a shortage of the photosynthetic products, probably of sugars.

With regard to leaf number, the following facts were found. In the cases in which continuous darkness was intercepted with the continuous illumination for 10 days or more, the number of leaves produced on the main axis before flower initiation were almost equal to those observed at the bending of light interception, i.e. the plant seems to cease differentiating new leaf primordia on the shoot apex immediately after exposure to light and to begin initiating flower primordia.

In the case in which continuous darkness was intercepted with the continuous illumination for 5 days, the growth stage from the fifth to the tenth day after germination was most sensitive to photoperiods, and a gradual decrease in sensitivity was observed with delaying exposure to the light. The decrease in sensitivity may be due to a decrease in total metabolic activity caused by an insufficient supply of the nutrients in the prolonged dark culture.

Plants cultured in darkness for 50 days did not initiate flower buds on the main axes and the tillers which had little developed on account of the apical dominant

Table 5. Culture of Konosu No. 25 in total darkness, from December 11, 1956, to March 31 and to May 21, 1957.

Duration of dark culture in days	110	160
Main axis		
Flower initiation*	1/11	2/16
Flowering stage	1.0	1.5
Tiller		
Flower initiation**	0/8	5/23
Flowering stage	0.0	2.4

* Denominator: number of plants dissected.

Numerator: number of main axes with flower primordia.

** Denominator: number of tillers dissected.

Numerator: number of tillers with flower primordia.

growth of the main axis. But those cultured for 160 days initiated flower primordia on the tillers to some extent, and rarely on the main axes (Table 5).

Summary

1) The spring wheat, Konosu No. 25, was cultured aseptically in test tube containing the modified White's medium under various light conditions.

2) The flowering and growth of spring wheat were depressed under the prolonged short days, and especially in darkness.

3) The photoperiods inserted in continuous darkness promoted flowering and growth. The photoperiod of 5 days was not enough for flowering, but that of 10 days was sufficient.

4) The early growth stage from the sixth to the tenth day after germination was most sensitive in photoperiodic response. The sensitivity decreased gradually with proceeding growth stages in dark culture.

5) The plants cultured in darkness for 50 days did not initiate flower primordia, but those cultured for 160 days initiated on the tillers and rarely on the main axes.

I am much indebted to Prof. S. Imamura of Kyoto University for his much criticisms for this paper.

References

- 1) Tashima, Y., Proc. Japan Acad. 29: 271 (1953). 2) —, Mem. Fac. Agr. Kagoshima Univ. 2: 1 (1956). 3) —, and Kimura, K., *ibid.* 3: 59 (1958). 4) —, and Imamura, S., Proc. Japan Acad. 29: 581 (1953). 5) Haupt, W., Zeitschr. Bot. 42: 125 (1952). 6) Sugino, M., Bot. Mag. Tokyo 70: 369 (1957).

摘 要

和田清美：無菌培養における春まき小麦の花芽形成について

I. 明期の効果と感光期

春まきコムギ 鴻巣 25 号) を試験管内で無菌培養を行ない、種々の光条件下、特に暗黒下での培養に与えられた明期の花芽形成と生長におよぼす影響を調べた。(1) この植物の花芽形成および生長は、連光下で促進され、短日下で低下し、暗黒化では特に抑えられる。しかし、全暗黒下でも花芽形成能を有することが示された。たとえば、連光下では発芽後 20 日にして出穂を始めるが、暗黒下での培養では、50 日にも発芽がまったく見られず、まれではあるが 160 日でようやく主軸(莖頂)に花芽をみる。またこの時期には分けつした枝にやや多くの花芽を見ることができる。(2) 暗黒下での培養の途中で明期を与えた場合、(生育時期にかかわらず)、5 日間連続する明期は花芽形成に不十分であったが、10 日間の明期は十分であることを見た。(3) 暗黒下での培養の各生育時期に与えられた 5 日間連続する明期の花芽形成におよぼす効果は、それが与えられる生育時期で異なる。その結果からみて発芽後 5~10 日が明期に対してもっとも強く反応する生育時期と考えられる。そして、暗黒下での培養が長びくにつれて、明期の効果は減ずることが観察された。(静岡大学文理学部生物学教室)

Paper-chromatographic Studies on Change in Gibberellins during Seed Development and Germination in *Pharbitis Nil*

by Yutaka MURAKAMI*

Received December 13, 1960

The occurrence of gibberellin-like substances in higher plants has been observed by several investigators including the author with various plant extracts¹). Also it has been known that immature seeds contain these substances of much higher concentrations than those in mature seeds of the same species¹). In a previous paper²) the author has reported that the paper-chromatographic behaviors of active materials from several kinds of immature seeds differ from those of the chemically known gibberellins. The work here described was done to see whether or not the paper-chromatographic pattern of gibberellin activity remained unchanged during seed development and germination. The use of the Japanese morning-glory as plant material is very convenient for this purpose, since its seeds are extremely abundant in gibberellin-like substances^{3,4}).

Materials and Methods

Seeds of *Pharbitis Nil* (Japanese morning-glory) were sown in pots and grown in a greenhouse at about 25°. From these plants, fruit samples were taken for the first time on the 6th day after anthesis and samplings were continued until the maturity of the seeds at the specified intervals. Each fruit usually contains 4 seeds and so 4 seeds or seedlings were used for the measurements of gibberellins.

For studies of seedlings the seeds were germinated and permitted to develop on a moist filter paper at about 20° in the dark room. Four seedlings were taken at the specified intervals.

Four seeds or seedlings, immediately after harvest, were weighed, ground with a pestle and mortar, extracted with 50 ml. of 70% acetone for 20 hours at room temperature, and then filtered. The residue was extracted once more in a similar manner for 3 hours. The combined filtrate was evaporated to dryness under reduced pressure. The resulting residue was dissolved in a small volume of 70% acetone and directly used for paper chromatography to separate the various active substances present in the extracts.

A sheet of Toyo No. 50 filter paper was used throughout the work. Chromatograms of the extracts were developed by the ascending method in the dark at about 27°, until the solvent front reached 30 cm. from the starting line. The solvent used was the mixture of *iso*-propanol/ammonia/water (10 : 1 : 1 V/V). The developed chromatogram was dried, folded lengthwise 2 cm. in width, and cut transversely at 2 cm. intervals. Each segment was then placed in beakers 2 cm. in diameter and 7 cm. in height containing 1.5 ml. of water. The author's rice seedling method was used to detect gibberellin activity. Five rice seedlings "Aichi-Asahi", whose coleoptiles attained about 1 mm., were planted in each beaker and allowed to grow under ordinary

* National Institute of Agricultural Sciences, Nishigahara, Kita-Ku, Tokyo, Japan.

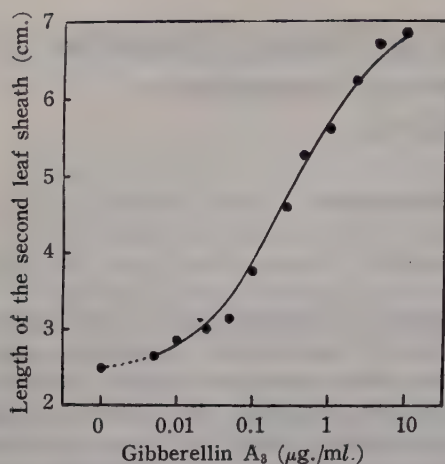


Fig. 1. Response of the second leaf sheath of rice seedling to gibberellin A₃.

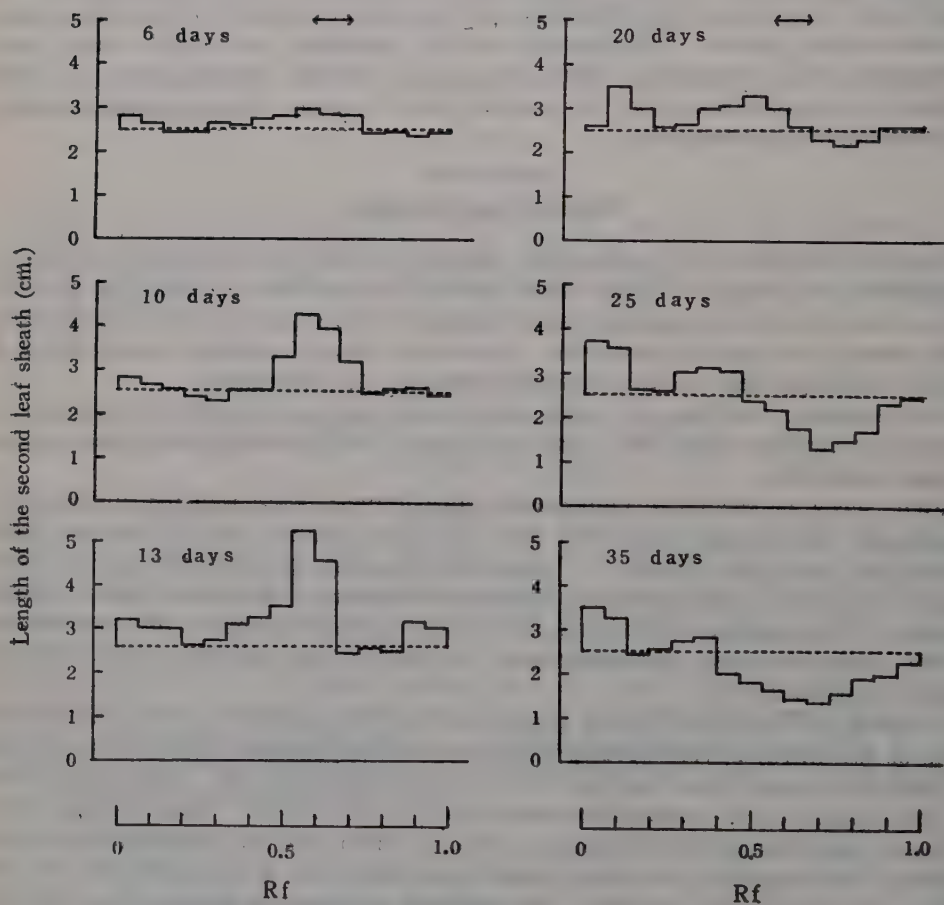


Fig. 2. Histograms showing gibberellin activity of acetone extracts of 4 seeds of *Pharbitis Nil* from anthesis to maturity after paper-chromatographic development with ammoniacal *iso*-propanol. Days on the histograms represent the time after anthesis. Broken lines denote water controls. Arrows at the top of the histograms indicate the position of gibberellin A₃.

daylight conditions at about 30°. They were supplied with 0.5 ml. water every other day. The length of the second leaf sheath was measured after 7 days when the third leaf blade was beginning to unfold from the second leaf sheath. The results were expressed in the mean length per beaker. A typical growth response of the second leaf sheath of rice seedlings to gibberellin A₃ is shown in Fig. 1.

Results and Discussion

The histograms of gibberellin activity obtained with acetone extracts of the seeds of *Pharbitis Nil* at different stages of maturity are shown in Fig. 2. This figure is based on the experiment in which the bioassay was made with rice seedlings. Therefore the term gibberellin is used for compounds having the property of causing growth promotion of the second leaf sheath of rice seedlings. The horizontal broken lines in the figure represent the growth of controls so that leaf sheath elongations greater than this represent the presence of gibberellin activity.

Changes in the level of gibberellin during seed maturity, which were determined by the methods indicated in Figs. 1 and 2, are presented in Fig. 3. In this figure the changes in fresh weight of seeds are shown. The results, which are evident from Fig. 3, show that under the conditions of present experiments the amount of gibberellin rises rapidly after anthesis to 13th day, but thereafter it falls markedly. The amount extracted from the immature seeds on 13th day after anthesis corresponds to 1.4 μ g. of gibberellin A₃ per 4 seeds. When the seeds were fully ripened, it decreased to only 1/6 of what it was on the 13th day after anthesis. On the 13th day after anthesis the embryo in the seed was as small as 2 mm. long but on the 20th day it was as large as the seed 10 mm. long. The maximum growth of the seeds, expressed in fresh weight increase, appeared subsequent to the time when the amount gibberellin reached the highest level. These results are in agreement with those of the earlier report of Mitchell *et al.*⁵⁾ and also of Corcoran⁶⁾ with *Echinocystis macrocarpa*, *Lupinus succulentus*, and *Phaseolus vulgaris*.

The occurrence not only of quantitative but also of qualitative changes in gibberellin during the development of *Pharbitis Nil* seeds can be seen on the histograms in Fig. 2. Until 13th day after anthesis the pattern of growth-promoting activity remained almost unchanged qualitatively. The growth-promoting zone of Rf 0.55–0.7 contained the major growth substance which promoted a remarkable elongation of rice seedlings and reached a maximum on the 13th day after anthesis. Since this promoting zone occurs at the Rf similar to that of gibberellins A₁, A₂, A₃ and A₄, one of which, gibberellin A₁, has been isolated from higher plants^{7,8,9)}, the growth

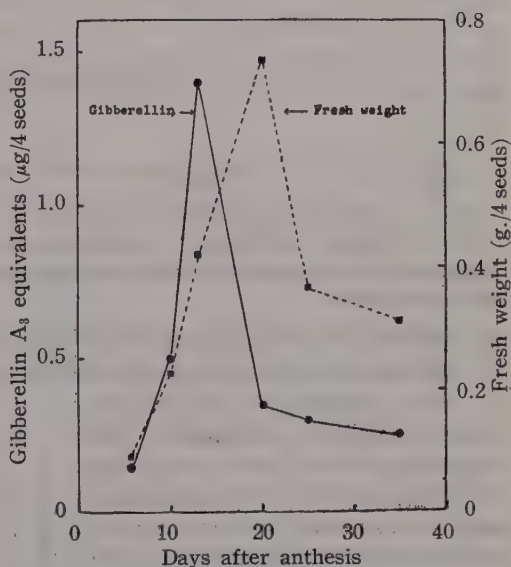


Fig. 3. Changes in gibberellin concentration and fresh weight in *Pharbitis Nil* seeds from anthesis to maturity.

promotion in this zone is considered to be due to the presence of gibberellin A₁, A₂, A₃ or/and A₄. Some indications of small activity were also found in other zones of the chromatograms.

On the 20th day after anthesis the level of gibberellin in the R_f region of 0.55–0.7 suddenly decreased, but two other promoting zones, which have smaller R_f values, 0.0–0.2 and 0.3–0.5, respectively, appeared. Moreover, a large inhibiting zone appeared in the region between R_f 0.6 and 0.9. And the pattern of growth substances remained unchanged until seed maturity.

It was found from preliminary experiments that the inhibiting substances are able to be extracted from an aqueous solution with chloroform at pH 2.5 but under the same conditions gibberellin A is not extractable. Then the syrup obtained from 4 mature seeds as described above, was taken up with 30 ml. water, acidified to pH 2.5 with phosphoric acid and extracted two times with the equal volume of chloroform. This chloroform extract was discarded. After the pH of the aqueous fraction was adjusted to 7.0 with 10% potassium hydroxide, the solution was evaporated to a small volume under reduced pressure, separated on paper chromatogram

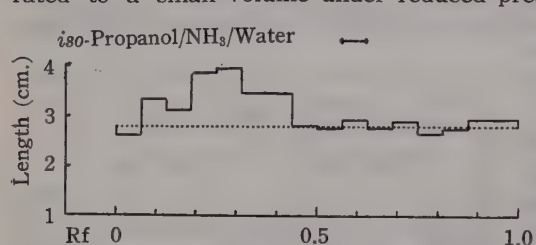


Fig. 4. The histogram showing gibberellin activity of acetone extracts of 4 mature seeds of *Pharbitis Nil* after removing inhibiting substances with chloroform.

the starting line of the chromatogram is attributable to the inhibition of rice seedlings by the high concentration of salts which was derived from neutralizing the eluate.

Since impurities in the extracts cause variation in both the R_f and the tailing of the active substance on chromatograms, it seemed necessary to examine further the chromatographic behavior of these active substances in the region of R_f 0.0–0.5. Then 40 *Pharbitis Nil* seeds at later stages of development were extracted with 70% acetone and the extract was chromatographed on a sheet of paper with ammoniacal *iso*-propanol as in the preceding experiment. The zone corresponding to R_f 0.0–0.5 was eluted with boiling 100 ml. of 50% ethanol three times, the eluate dried, taken up into a small amount of water, and chromatographed ascendingly with

with ammoniacal *iso*-propanol, and bioassayed by the rice seedling method. The result is given in Fig. 4. On the chromatogram in Fig. 4 no promoting zone corresponding to gibberellin A₃ occurred. Of course, as already reported in a previous paper³), the promoting zone corresponding to gibberellin A₃ could be clearly detected in the extract when more than 10 mature seeds of *Pharbitis Nil* were used. The disappearance of growth promotion at

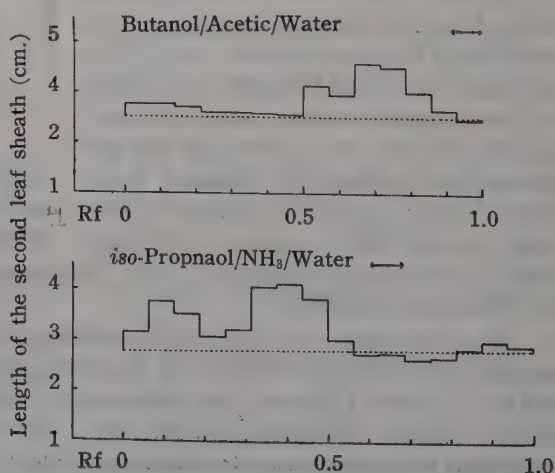


Fig. 5. Histograms showing gibberellin activity of acetone extracts of mature *Pharbitis Nil* seeds. Details are described in the text.

n-butanol/glacial acetic acid/water (4 : 1 : 2 V/V) at 27°. After the chromatogram was divided lengthwise into three strips, one of them was cut into 14 equal sections according to the R_f values and bioassayed by the rice seedling method. The result is shown in Fig. 5. A broad growth-promoting zone was found at R_f 0.5–0.85. Gibberellin A_3 runs with the solvent front under the same conditions.

From the remaining strips the zone between R_f 0.5 and R_f 0.85 was excised, re-extracted with boiling 50% ethanol, and the extract again paper-chromatographed with ammoniacal isopropanol, and bioassayed as above. It was found that the promoting activity was reproduced in the zones of R_f 0.0–0.2 and R_f 0.3–0.5 (Fig. 5). From these results it is confirmed that *Pharbitis Nil* seeds contain new gibberellins whose paper-chromatographic behavior is distinguished from that of the chemically known gibberellins A_1 , A_2 , A_3 and A_4 . Also none of them is identical with bean factor II or gibberellin A_5 , since bean factor II is reported to run two times as far as gibberellin A_3 in an ammoniacal *n*-butanol solution⁹).

The pattern of growth substances on the paper chromatogram obtained from the etiolated *Pharbitis Nil* seedlings is presented in Fig. 6. In the histograms of seedlings the same promoting and inhibiting zones are found as in later stages of seed development: two promoting zones at R_f 0.0–0.2 and R_f 0.3–0.5, and a broad inhibiting zone at R_f 0.6–0.9. The promoting zone corresponding to gibberellin A_3 could not be detected with 4 etiolated seedlings between 2 and 14 days after germination. The level of gibberellin extracted from the etiolated seedlings, which was determined

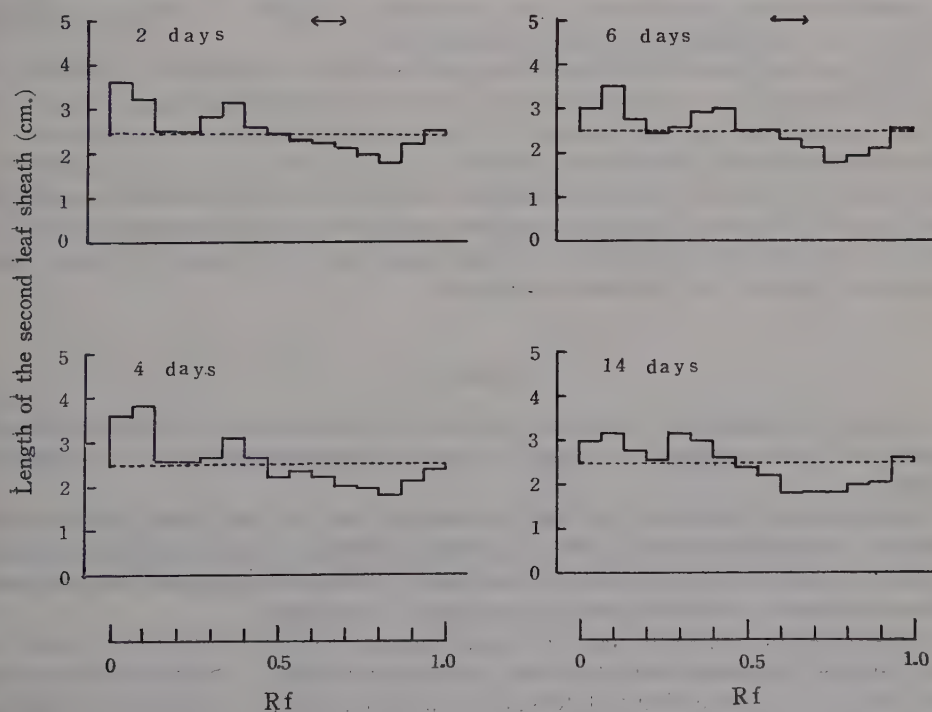


Fig. 6. Histograms showing gibberellin activity of acetone extracts of 4 etiolated seedlings of *Pharbitis Nil* after paper-chromatographic development with ammoniacal *iso*-propanol. Days on the histograms represent the time after germination. Further explanations are as in Fig. 2.

by Figs. 1 and 6, decreased gradually after germination in the dark as shown in Fig. 7. The similar pattern of growth substances was also found with 4 light-grown seedlings at the cotyledon stage (Fig. 8).

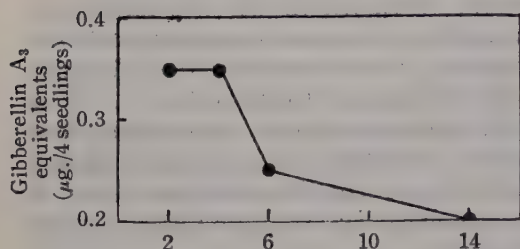


Fig. 7. Changes in gibberellin concentration in etiolated *Pharbitis Nil* seedlings after germination.

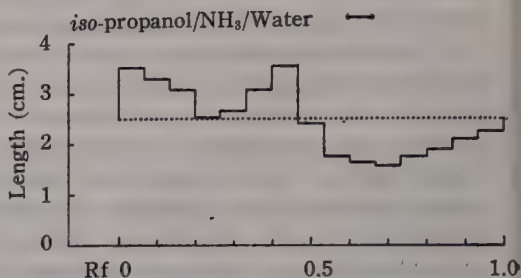


Fig. 8. The histogram showing gibberellin activity of acetone extracts of 4 light-grown seedlings of *Pharbitis Nil* at the stage having expanded cotyledons.

As already described, the growth-promoting zone corresponding to gibberellin A₃ suddenly disappeared at the later stage of seed development and instead two new promoting zones with smaller Rf values occurred. This phenomenon leads the author to suspect that gibberellin A₃ or similar compounds might be converted into these new growth-promoting substances in the plant tissue. In order to confirm this hypothesis further experiments are being carried out.

In a previous paper²⁾ the author has reported that two zones of gibberellin activity are shown on the chromatograms of immature seeds of leguminous plants and that the activity of the one with Rf 0.7 is attributed to the known gibberellin A₃, while that of the other with smaller Rf value of 0.2 is due to a new gibberellin. This smaller Rf value is practically identical with that of a new gibberellin extracted from *Pharbitis Nil* seeds. Judging from the results of present experiments, the occurrence of two gibberellin activities in leguminous seeds is thought to depend upon the degree of maturity of the seeds employed.

Summary

Changes in gibberellins occurring during seed development and germination of *Pharbitis Nil* were studied by means of paper-chromatography and rice seedling method.

The amount of gibberellin reached its peak on the 13th day after anthesis and thereafter markedly decreased. The maximum amount of extracted gibberellin corresponded to 0.35 μg. of gibberellin A₃ per seed. Gibberellin extracted from etiolated seedlings decreased gradually after germination in the dark.

Three zones of gibberellin activity were found at Rf 0.0-0.2, Rf 0.3-0.5, and Rf 0.55-0.7 on the chromatograms developed with the mixture of *iso*-propanol/ammonia/water (10 : 1 : 1). The activity of Rf 0.55-0.7 was attributed to the chemically known gibberellin A₁, A₂, A₃, or A₄.

In initial stages of seed development the gibberellin activity appeared mainly at Rf 0.55-0.7, while in mature seeds and seedlings at smaller Rf values of 0.0-0.2 and 0.3-0.5.

The author wishes to acknowledge with thanks the encouraging interest of Dr. T. Hayashi.

References

- 1) Phinney, B. O., and West, C. A., *Ann. Rev. Plant Physiol.* **11**: 411 (1960).
- 2) Murakami, Y., *Bot. Mag. Tokyo* **72**: 36 (1959).
- 3) —, *ibid.* **72**: 438 (1959).
- 4) Ogawa, Y., and Imamura, S., *ibid.* **73**: 125 (1960).
- 5) Mitchell, J. W., Skaggs, D. P., and Anderson, W. P., *Science*. **114**: 159 (1951).
- 6) Corcoran, M. R., Cited by Phinney and West, *Ann. Rev. Plant Physiol.* **11**: 411 (1960).
- 7) MacMillan, J., and Suter, P. J., *Naturwiss.* **45**: 46 (1958).
- 8) Kawarada, A., and Sumiki, Y., *Bull. Agr. Chem. Soc. Japan* **23**: 343 (1959).
- 9) West, C. A., and Phinney, B. O., *J. Am. Chem. Soc.* **81**: 2424 (1959).

摘 要

村上 浩： アサガオの種子の成熟および発芽過程におけるジベレリンの消長

アサガオの開花から種子の成熟する過程と暗所にて種子を発芽させた場合におけるジベレリンの消長を、ペーパークロマトグラフィーにイネ苗試験法を併用して研究した。

アセトン抽出物は、アンモニア性イソプロピルアルコールによる展開で、A ($R_f=0-0.2$), B ($R_f=0.3-0.5$), C ($R_f=0.55-0.7$) の伸長促進部と、 $R_f=0.6-0.9$ の抑制部とにわかれた。C は馬鹿苗病菌の生産するジベレリンに相当する。種子の成熟とともに C が減少し、A, B が現われ、同時に抑制物質が出現する。芽ばえ抽出物のペーパークロマトグラムにおける作用物質のヒストグラムは完熟種子のそれと類似している。

成熟過程の種子のジベレリン含量は、開花後 13 日までは増加し (最高 1 種子当たり $0.35 \mu\text{g}$ のジベレリン A_3 に相当)、その後減少する。また発芽の場合、黄化苗では日の経つにつれてじょじょに減少した。

(農林省農業技術研究所)

Fine Structure of Diatom Valves III.

by S. C. MEHTA* and G. S. VENKATARAMAN**

Received December 13, 1960

The present paper deals with the fine structure of four centric diatoms, viz., *Hemidiscus hardmannianus* (Grev.) Mann, *Stephanopyxis palmeriana* (Grev.) Grun, *Skeletonema costatum* (Grev.) Cleve, and *Coscinodiscus concinnus* W. Smith. These diatoms were collected from Cape Comorin (Venkataraman, 1959¹) and from the estuarine regions of the river Hoogly by Mr. N. Dutta. The materials were cleaned as described earlier (Venkataraman and Mehta, 1960²) and observed under a Philips Electron-microscope (Mehta, under publication³).

Hemidiscus hardmannianus (Grev.) Mann (Figs. 1–8).

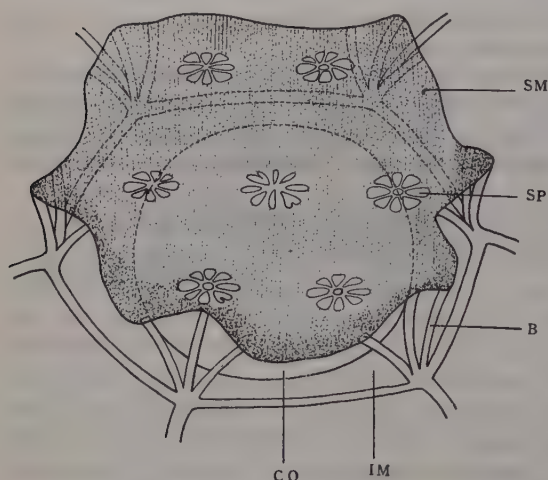


Fig. 1. Diagrammatic representation of an oblique view of the areolae of *Hemidiscus hardmannianus*. B, bars; CO, central opening; IM, inner membrane; SM, sieve membrane; SP, sieve pores.

tripod-like structure are the only supporting structures between the two membranes. The inner membrane has a single large central opening (CO) in each hexagon. The outer membrane has 4–8 groups of sieve pores (SP) per hexagon (Figs. 6 and 8). Around the margin there are spinulae (S) from which arise hyaline ribs (Fig. 4). The girdle wall is perforated by a series of simple pores arranged radially (Fig. 7). In its fine structure, this diatom resembles *Biddulphia mobliensis* Bailey (Cassie and Bertand, 1960⁴), although Okuno described simple areolae with round sieve pores in this form (Okuno, 1949b⁵).

*L.M.S.**** Valves semicircular with straight ventral margin (Fig. 2). Ends are obtuse and the central portion is large and hyaline. The areolation is fine and radiating from the centre, about 10–12 in 10 μ . Spinulae are seen around the margin with hyaline ribs arising from them and running to the centre.

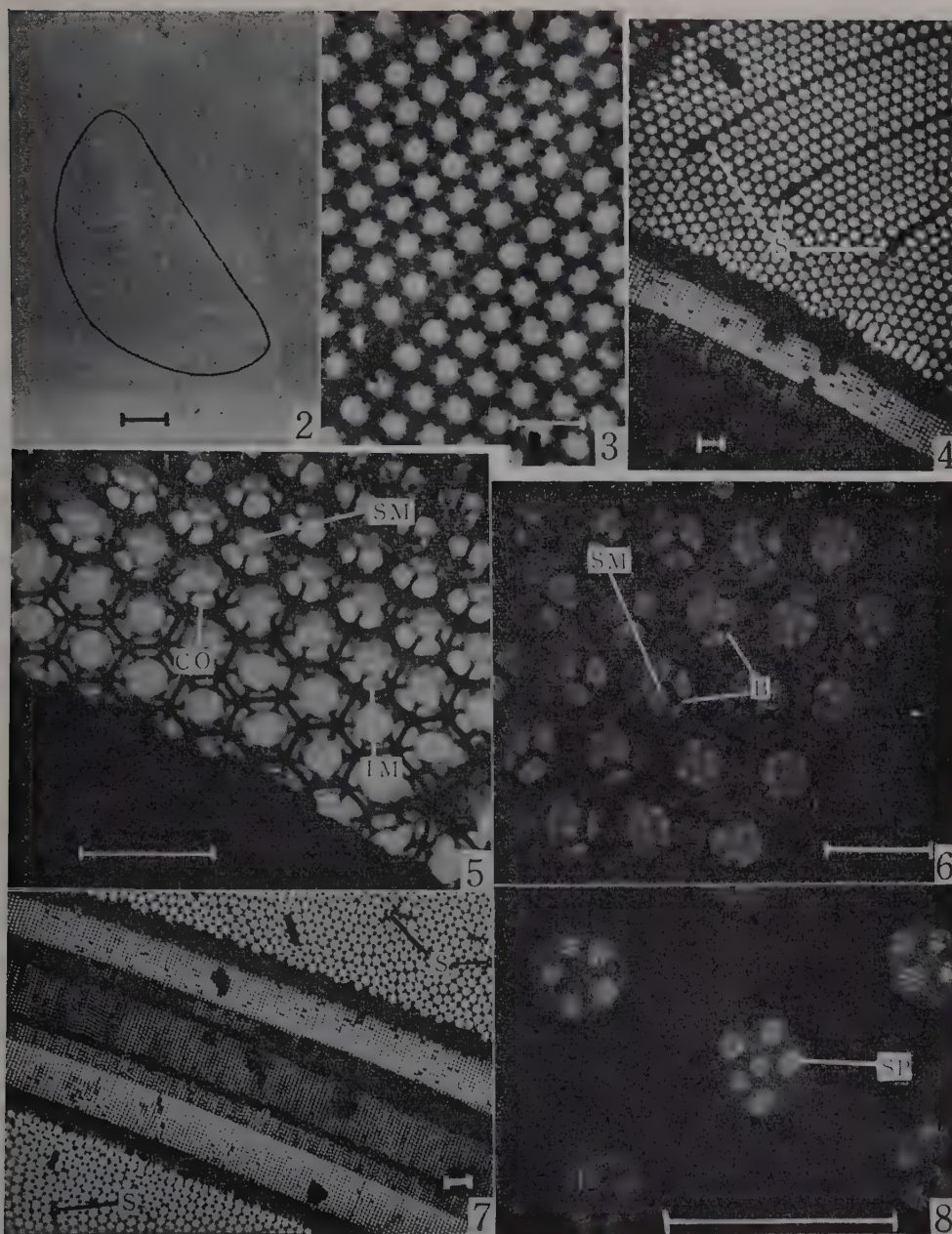
*E.M.S.***** Under the electron microscope, the valve resolves itself into a lattice of hexagons with two distinct membranes (Figs. 1, 5 and 6). The inner membrane (IM) is supported by a series of hexagons. From each angle of the hexagon, three curved bars (B) project out, on which rests the outer sieve membrane (SM) (Figs. 5 and 6). It seems that there are no lateral walls and these projecting bars forming a

* Division of Soil Science and Agricultural Chemistry, Indian Agricultural Research Institute, New Delhi-12.

** Division of Botany, Indian Agricultural Research Institute, New Delhi-12.

*** L.M.S. Light microscope study.

**** E.M.S. Electron microscope study.



Figs. 2-8. *Hemidiscus hardmannianus*. Fig. 2, valve view. Figs. 3, 4, part of the valve showing 'hyaline rib lines' (note the spinulae in Fig. 4). Figs. 5, 6, portions of the valve viewed obliquely. Fig. 7, portion of the girdle. Fig. 8, portion of the valve viewed vertically (note the groups of sieve pores) (Fig. 2, lucida picture, scale: 10μ . Figs. 3-8, electron-micrographs, scale: 1μ). B, bars; CO, central opening; IM, inner membrane; SM, sieve membrane; SP, sieve pores; S, spinulae.

Stephanopyxis palmeriana (Grev.) Grunn (Fig. 14).

E.M.S. The fine structure of this diatom has been studied by Desikachary and Bahadur (1954⁶) and Okuno (1955⁷), but the girdle structure was not figured by them. The girdle in this diatom resembles that of *Stephanopyxis orbicularis* (Cassie and Bertrand, 1960⁸). The girdle wall is single layered and perforated by a series of simple pores. The radial arrangement of the pores is interrupted by nonperforated bands (Fig. 14), which simulate the appearance of the intercalary bands in *Rhizosolenia*.

Skeletonema costatum (Grev.) Cleve (Figs. 9-13).

L.M.S. Frustules lens-shaped with rounded ends, and joined by spines by silicification. The space between the cells is longer than the cells. The cells are 11.8-16.3 μ in diameter. No visible structure could be observed on the valve under the optical microscope.

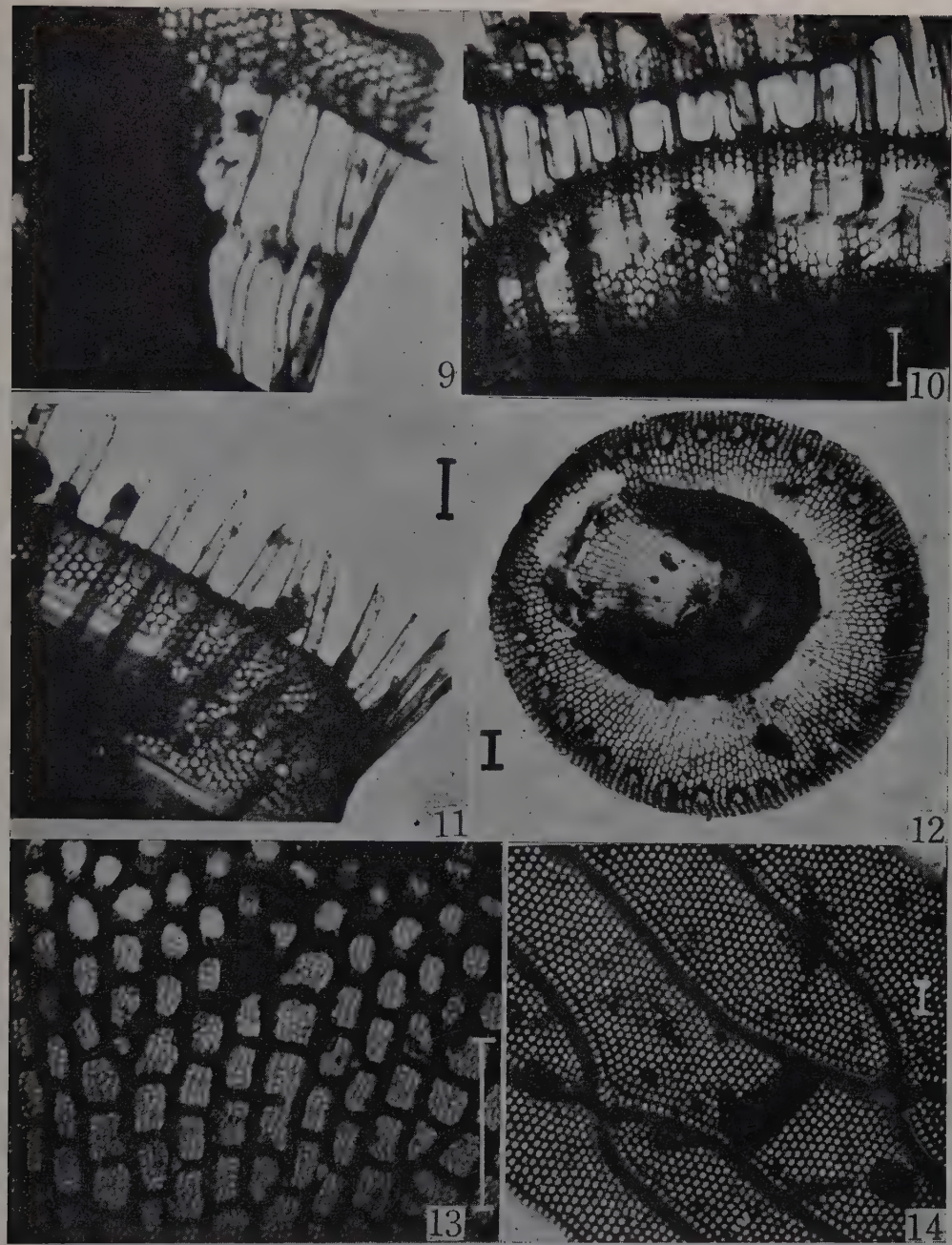
E.M.S. The wall structure of this diatom, commonly known as 'perivalvar punctiert-gestreift' (Hustedt, 1930⁸) has been studied by Kolbe (1948⁹) and Desikachary and Bahadur (1954⁶). The valve shows clear areolae formation as reported by Kolbe (1948⁹) and Desikachary and Bahadur (1954⁶) and the areolae are of the fully open type with secondary areolae with poroid membrane (Figs. 12 and 13). The spines are hollow and nonperforated (Figs. 9-11) and are firmly joined by silicification (Fig. 9). It was doubted whether the secondary areolae formed a separate layer or not (Desikachary and Bahadur, 1954⁶). Fig. 12 shows the complete secondary areolae from inside with the bases of the spines all around. This shows that they form an independent layer, which is closely attached to the outer primary one. It thus confirms that in *Skeletonema costatum*, the secondary areolae are formed as an independent layer closely adpressed to the primary layer by silicification and not as a more closing membrane (Desikachary and Bahadur, 1954⁶).

Coscinodiscus concinnus W. Smith (Figs. 15-21).

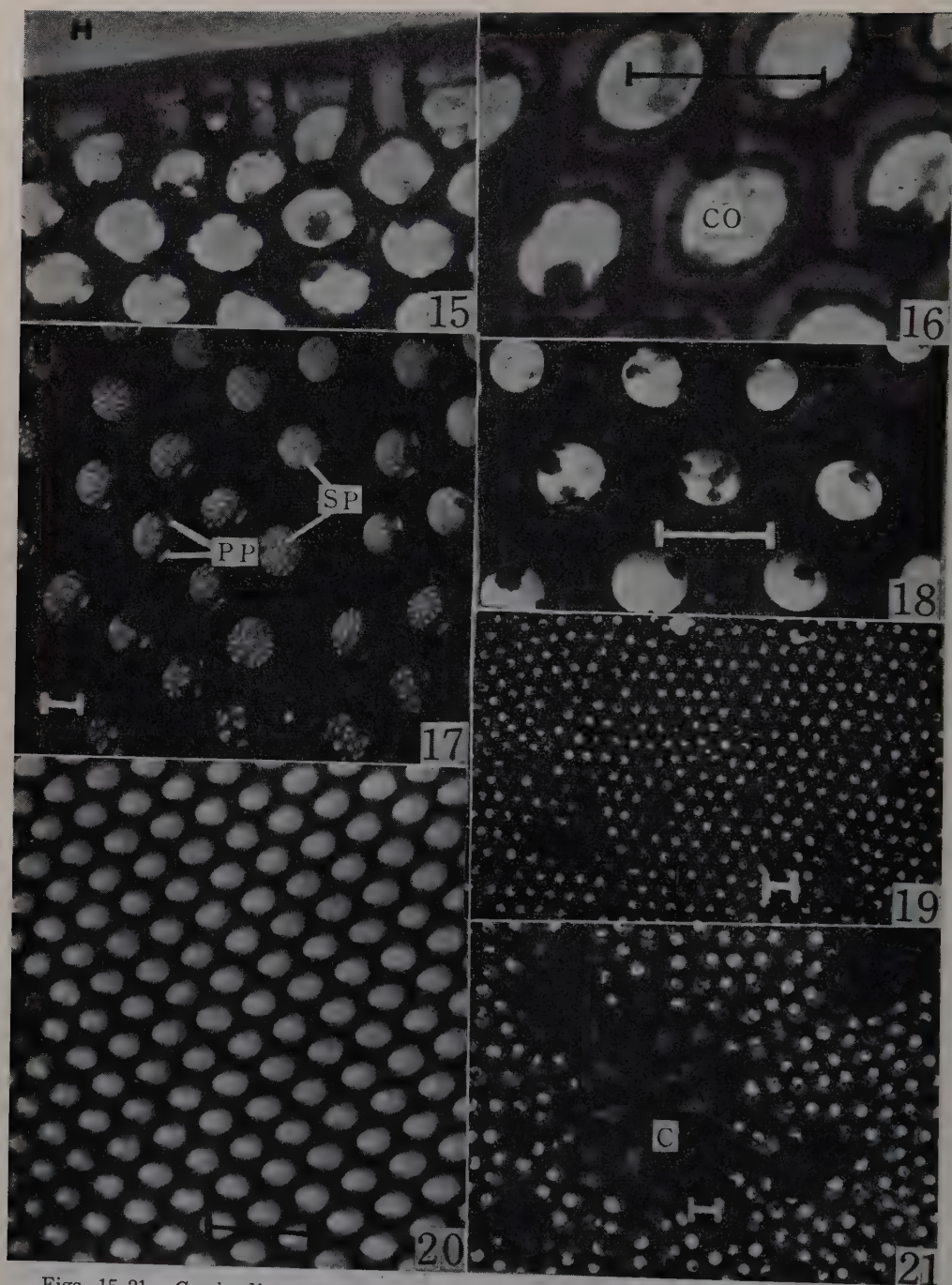
L.M.S. The frustules are large, drum-shaped, 200-420 μ in diameter. Valve surface is slightly convex and in the centre sometimes slightly depressed. Areolation is slender with well differentiated rosette of large meshes, the surrounding areolae becoming suddenly smaller, about 9-12 in 10 μ at the centre and 12 in 10 μ near the margin; central rosette distinct, radial; secondary series regular, hyaline ribs running to the centre from distinct spinulae near the margin; two small asymmetrical processes clearly discernible.

E.M.S. The areolae on the valve are regularly hexagonal in pattern and are open to the inside, each side of the loculus being 0.4-0.6 μ long. Each loculus is provided with an outer sieve membrane, an inner closing membrane and lateral membranes. The sieve membrane possesses many polygonal sieve pores (SP) with somewhat irregular margins. The sieve pores are 9-12 in 1 μ and more or less in concentric rows. The inner closing membrane has a round central opening (CO). The lateral membrane is six-sided and the neighbouring loculi communicate with each other by means of passage pores (PP) on the lateral membrane (Figs. 17 and 19). The girdle loculi are arranged in longitudinal and oblique rows (Fig. 20). The loculi are with outer and inner openings, the outer opening lacking the closing sieve membrane (Okuno, 1955⁷). The present observations confirm those of Okuno (1955⁷). In *C. elegans* and *C. pseudonitidulus* the sieve membrane is non-porous (Okuno, 1953c¹⁰) and 1954d¹¹).

The authors wish to express their thanks to Drs. M. S. Randhawa, B. P. Pal, A. B. Joshi and R. V. Tamhane for their interest and encouragement, to Drs. C.



Figs. 9-14. *Skeletonema costatum*. Figs. 9-11, spines. Fig. 12, secondary areolar layer viewed from inside. Fig. 13, secondary areolae with poroid membranes. Fig. 14, *Stephanopyxis palmeriana*, girdle view (scale: 1 μ).



Figs. 15-21. *Coscinodiscus concinnus*. Fig. 15, margin of the valve. Figs. 16-18, portions of the valve. Fig. 19, part of the valve near the central area showing the 'hyaline lines'. Fig. 20, portion of girdle viewed obliquely from inside. Fig. 21, central rosette (scale: 1μ). C, central rosette, CO, central opening; PP, passage pores; SP, sieve pores.

Dakshinamurti and T. V. Desikachary for their advice and to Mr. N. Dutta for kindly placing some of his collections at our disposal.

References

- 1) Venkataraman, G. S., Proc. Natl. Inst. Sci. India **24b**: 307 (1959). 2) —, and Mehta, S. C., Phytomorph. **10**: 116 (1960). 3) Mehta, S. C., Sci. and Culture (under publication). 4) Cassie, V., and Bertand, W. S., J. Zoy. Micr. Soc. **79**: 89 (1960). 5) Okuno, H., Bot. Mag. Tokyo **62**: 136 (1949b). 6) Desikachary, T. V., and Bahadur, K., Trans. Amer. Micr. Soc. **73**: 274 (1954). 7) Okuno, H., Bot. Mag. Tokyo **68**: 125 (1955). 8) Hustedt, F., Die Kieselalgen in Rabenhorst's Kryptogamenflora, Bd. 7, Akad. Verlagsges., Leipzig (1930). 9) Kolbe, R. W., Ark. Bot. **33**: 1 (1948). 10) Okuno, H., Bot. Mag. Tokyo **66**: 121 (1953c). 11) —, Trans. Proc. Palaeont. Soc. Japan, N. S., **13**: 125 (1954d).

湖水の循環期および停滞期における水生菌類 の遊走子の垂直分布

鈴 木 静 夫*

Shizuo SUZUKI*: The Vertical Distributions of the Zoospores of Aquatic Fungi during the Circulation and the Stagnation Periods

1960 年 6 月 28 日受付

湖沼の生物の垂直分布はきわめて興味ある問題であり、特にプランクトン類については多くの研究がなされ、その概要が明らかになった。しかし、微生物群に関しては、細菌類を除いてほとんど見るべき研究がなされていない。特に湖沼に棲息する水生菌類の遊走子は2本または1本のべん毛をもつて水中を遊泳できるので、動物プランクトンと同様にその垂直分布には興味をもたれる。著者は湖沼における水生菌類の生態学的研究の一環として、富栄養型に属する数個の湖沼において、水生菌類の遊走子の垂直分布と生態的要因との関係について観察を行なつたので、その結果を報告する。

研 究 方 法

採水はエクマン式顛倒採水器を使用して、湖の中央部で1mあるいは2m間隔で表層から水底までの各層について行なつた。水温は顛倒寒暖計によつて測定し、同時に溶存酸素量を Winkler 法によつて定量した。

採水した試水を滅菌したビンかポリエチレンの袋に入れて実験室にもち帰り、試水を十分に攪拌したのちに35mlを滅菌したシャーレに取る。このなかによく似たアサの実を半分に割ったものを3個ずつ水面に浮かす。シャーレは室温に数日間放置し、ア

サの実の上に発生した菌糸数によって、試水中の水生菌類の遊走子数を推定した。遊走子数の計測は菌糸の太い *Saprolegnia*, *Isoachlya*, *Achlya*, *Dicetyuchus*, *Thraustotheca*, *Aplanes* の各属だけに行ない、菌糸が細く、分岐の多い *Aphanomyces* 属と *Pythium* 属は計測から除外した。菌糸数を数え終わったアサの実を滅菌水中でさらに数日間培養し、生殖器官の形成ののちに種の同定を行なった。

観 察 結 果

1. 遊走子の垂直分布

柳沼、中沼、震生湖などの深い富栄養湖では、夏季停滞期には躍層を境に、その上層と下層とでは水の理化学的な性質が全く異なる。このことは特に溶存酸素量にいちじるしく、表水層には十分に溶存しているが、躍層で急激に減少し、深水層は無酸素状態となっている。この時期の遊走子の垂直分布は第1図に示すように、遊走子は表水層にだけ分布し、酸素の消失した深水層にはまったく見られない。特に水温の上昇した7月と8月には、遊走子の分布は表層に限られる。Salvin¹⁾は水生菌類の遊走子は、水温が20~27°では活発に運動するが、それ以上の水温ではほとんど運動が行なわれなことを観察している。夏季停滞期には表面の水温は27~30°になるので、遊走子はほとんど活動することができず、休止の形で水面に浮いていると考えられる。同様な事実は、腐植栄養型に属する大峯沼でも観察され、湖沼型による差異はないようである。

* Department of Microbial Chemistry, Faculty of Pharmacy, Tokyo College of Science, Tokyo, Japan. 東京理科大学薬学部微生物化学教室。

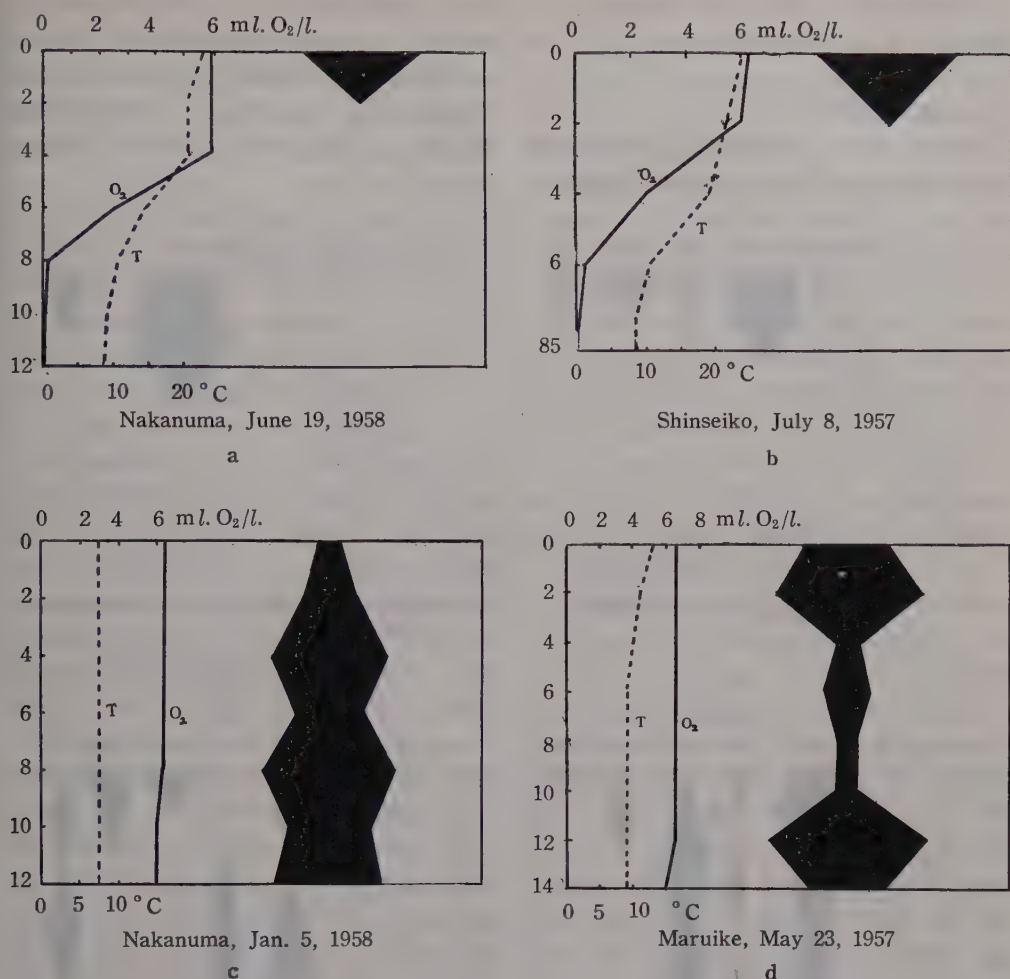


Fig. 1. Vertical distributions of zoospores of aquatic fungi during the stagnation and circulation periods. (Depth in m.)

中沼をはじめ二、三の湖沼で、非常にまれではあるが、停滞期においても水生菌類の遊走子が無酸素状態にある湖底直上の水に分布していることが観察された。また、無酸素水のなかで水生菌類を培養すると、まれに遊走子が形成される²⁾。この事実、停滞期においてもまれに湖底泥中で遊走子の形成が起こり、放出された遊走子は無酸素のために遊泳できずまた水が停滞しているので他層へ運ばれないので水底に分布したものと考えられる。

循環期においては、溶存酸素量および水温ともに表層から深層まで一様である。水生菌類の遊走子は

第1図に示すように、各湖沼とも表層から深層までの各層に見られ、量的にも非常に多い。これは湖水がよく循環するために、沿岸部および湖底泥中で形成された遊走子が、各層に運ばれたためと考えられる。しかしながら、丸池や切所沼の例に見るように、湖底直上の水に特に遊走子が多く見られることがあるが、これは湖底泥中で遊走子が形成されるためであろう。

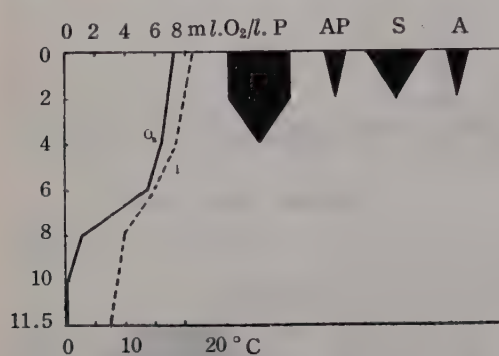
丸池の循環期から停滞期に入る時期（5月23日）には、遊走子は表層と底層に非常に多く、中層には少ない。同様な現象は他の二、三の湖沼でも観察され、

一般に循環期の均等分布から停滞期の層状的の分布に移る場合、まず中層の遊走子が消失するようである。

2. 種類の垂直分布

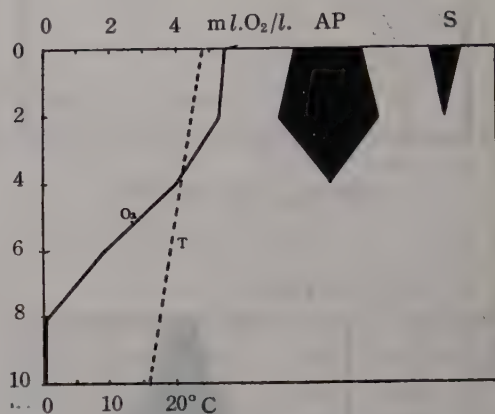
水深の深い湖沼における循環期および停滞期の各種類の垂直分布は第2図に示す通りで、停滞期には

深層水の酸素が消失するので、すべての種類は表水層に分布し、特に夏季にはこの傾向がいちじるしい。震生湖の観察では、停滞期にも *Pythium* sp. が無酸素状態の水底に少数見られた。これは本種が無酸素の状態にある湖底泥中でもなお遊走子を形成するためであろう。



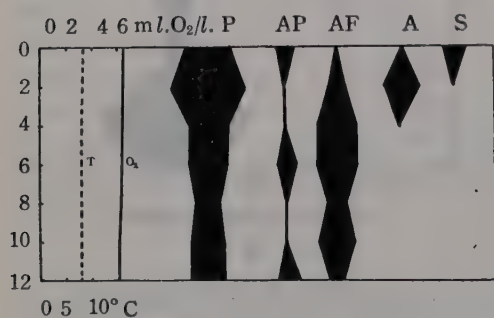
Nakanuma, May 24, 1958

a



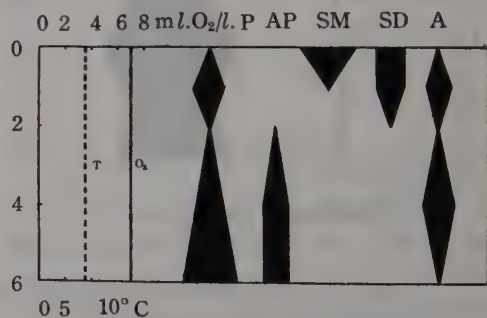
Yanaginuma, Aug. 21, 1957

b



Nakanuma, Jan. 5, 1958

c



Yanaginuma, May 5, 1957

d

Fig. 2. Vertical distributions of some species of aquatic fungi during the stagnation and circulation periods. (Depth in m.)

P : *Pythium* sp.

SM : *Saprolegnia monoica*

S : *Saprolegnia* sp.

A : *Achlya* sp.

AP : *Aphanomyces* sp.

SD : *Saprolegnia diclina*

AF : *Achlya flagellata*

循環期においては、湖水の理化学的な性質が表層から深層までほとんど一様であるにもかかわらず、水生菌類の垂直分布は種類によつて異なっている。すなわち、湖沼に広く分布する *Saprolegnia monoica*, *Saprolegnia diclina* などの *Saprolegnia*

属は、表層に多く、中層と深層にはほとんど分布しない。 *Achlya americana*, *Achlya flagellata*, *Achlya racemosa* などは中層と深層に多く、表層には少ない傾向が見られた。また、 *Aphanomyces* 属は各層に分布するが、特に深層に多く認められた。

Pythium 属は湖沼によって多少異なるが、一般に表層に多い傾向が観察された。このような垂直分布の傾向は、水深が1m前後の浅い沼でも同じであった。

考 察

湖沼における水生菌類の遊走子の垂直分布を支配する要因として、溶存酸素量、湖水の循環あるいは停滞、水温などが考えられる。Salvin¹⁾によれば、水生菌類の遊走子は2または1本のべん毛をもち、毎秒100~300 μ の速さで自由に運動するという。しかし、この程度の運動力では、水深が10mあるいはそれ以上に達するような深い湖沼では、遊走子自身の運動が分布の決定的な要因とは考えられず、むしろ水の循環による遊走子の移動が大きな要因となっている。実際に完全な循環期には、遊走子は表層から深層まで分布していた。湖水がまったく循環しない夏季停滞期には、深層には水生菌類の遊走子は存在しない。たとえ停滞期に湖底泥中で遊走子形成が行なわれても深水層が無酸素なために、遊走子は休止の状態で水底にとどまり、短時間で死滅すると考えられる。

循環期に種類によって垂直分布が異なることは注目すべきことで、生態学的に興味ある問題である。*Saprolegnia* 属は表層に多い傾向が見られたが、シャーレ中で *Saprolegnia monoica* や *Saprolegnia diclina* の遊走子の分布を観察すると、遊走子はすべて水の表面に存在している。特に休止状態にある遊走子は水面に浮いており、本属の遊走子の比重が小さいためと考えられる。

水温の低い循環期には *Achlya* 属や *Aphanomyces* 属の遊走子が水底に多い (Fig. 2)。この原因として、遊走子が遊走子嚢から直接個々に泳出するのではなく、頂端の孔から出た遊走子は孔辺に球形の塊を作って休止する。もしも条件がわるいと、遊走子は長時間塊のままで水底に沈むために、深層に多い結果となったものと思われる。特に水温の低下した冬季にはこの傾向がいちじるしい。標本ビンを使用して水生菌類の遊走子の垂直分布を観察したところ、*Saprolegnia* 属は表層から水底まで一様に分布したが、*Achlya americana* は水底に多い傾向が見られ、湖沼における観察結果と一致している。

Cotner³⁾, Salvin¹⁾ などによると、水生菌類の遊走

子は水温が30°以上に上昇すると運動を行なわないというが、平地の富栄養湖では夏季には水温が30°にもなるので、遊走子はすべて休止状態にあるものと考えられ、その結果、水面に浮くものと推察される。

循環期から停滞期に移行する場合、遊走子は均一な分布からまず中層の遊走子が消失し、表層と深層にだけ分布する型が現われる。この原因として遊走子の生存時間が問題となる。著者の観察によれば、*Pythium* 属を除く水生菌類の遊走子の生存時間は2~3日のために、湖水の成層が始まる春季には水の循環が不完全なため、湖底泥中および沿岸部で形成された遊走子は中層に運ばれず、まれに運ばれたとしても短時間で死滅するので中層に消失するものと思われる。さらに成層が進むと水底は酸素がなくなり、泥中で遊走子の形成がほとんど起こらないので、遊走子は深層からも消失する。

水生菌類の遊走子は湖水中で明らかに二つの型があり、一つはべん毛を有して運動をする型、他の一つは膜をかぶって休止状態にある型である。この両形態は外界条件が良好なときには交互にくり返して生ずる。Höhnk⁴⁾, Salvin⁵⁾によると、運動期と休止期の時間は外界条件や種類によっても異なるが、通常おのおの約1~2時間でくり返されるという。湖沼においてもこの二形態によって分布の状態が異なることが予期されるが、シャーレに遊走子を入れて顕微鏡で観察すると、休止形のものは水面に浮か、あるいはシャーレの底に沈んでいるが、活動形の遊走子は種々の深さに分布している。これは湖沼における水生菌類の遊走子の垂直分布を解析していく場合、遊走子の運動形と休止形とを区別しなければならぬことを示しているが、これについては今後の研究にゆずる。

摘 要

湖沼での水生菌類の遊走子の垂直分布は、明らかに二つの型が存在する。一つは遊走子が表層から深層までほとんど均一に分布する型で、主として循環期に見られる。他の一つは遊走子が表層にだけ分布するもので、停滞期に見られる。このような分布型を決定する要因としては、遊走子自身の運動よりはむしろ湖水の循環や停滞が重要である。

循環期には種類によって垂直分布はかならずしも

均一ではなく、一般に *Saprolegnia* 属は表層にだけ分布するが、*Achlya* 属と *Aphanomyces* 属は底層に多い傾向が観察された。*Pythium* 属は表層から水底までの各層に一樣に分布するが、表層にやや多い傾向が見られる。停滞期には各種類とも表水層あるいは水面にだけ分布し、無酸素状態にある深水層にはまったく見られない。

この研究に対して、指導ならびに助言をいただいた東京教育大学の印東弘玄、伊藤 洋両教授および市村俊英講師に深く感謝の意を表したい。また野外作業に多大の助力をお願いした畠山忠史氏に感謝する。

文 献

- 1) Salvin, S. B., *Mycologia* 33: 592 (1941). 2) 鈴木静夫, 植雑 74: 30 (1961). 3) Cotner, F. B., *Amer. Jour. Bot.* 17: 511 (1930). 4) Höhnk, W., *ibid.* 20: 45 (1933). 5) Salvin, S. B., *Mycologia* 32: 148 (1940).

Summary

The vertical distributions of the zoospores of aquatic fungi were studied in some Japanese lakes. The distribution of the zoospores in lake waters varied with seasons. There were two main types, i. e. homogeneous distribution and stratum one. The former appeared mainly during the circulation period and the latter was observed during the stagnation period.

All species of aquatic fungi were distributed only in the epilimnion during the stagnation period, while some differences in the vertical distribution of the fungi were observed during the circulation period. The zoospores of *Saprolegnia* were distributed only in the surface layer, while those of *Achlya* and *Aphanomyces* were seen mostly in the bottom water. The zoospores of *Pythium* were distributed homogeneously from the surface to the bottom layers.

ウシグソヒトヨにおける菌傘の開閉性変異*

木村 勘二**・藤生みさ子**

Katsuji KIMURA** and Misako FUJIO**: Variation in the Expansibility of Pilei in *Coprinus macrorrhizus* form. *microsporus**

1960 年 11 月 14 日受付

四極性帽菌の一つであるウシグソヒトヨ *Coprinus macrorrhizus* form. *microsporus*¹⁾ の 2 核菌糸体はふつう、ペレイショ煎汁寒天培養基上で、ように正常な子実体を形成するものである。すなわち、2 核菌糸を試験管内の斜面培養基に植えて 30° に保つと、5~7 日後には培養基一面に菌糸が生長すると同時に子実体の原基が現われ始め、それらは一両日中に正常な子実体に発達して菌傘を開き、胞子を落とす (第 1 図)。

しかし、外観正常な野生子実体 *h* (1958 年 9 月大津市にて本郷次雄氏採集) の胞子から由来した 2 核菌糸体においては、子実体の原基が現われても生長を途中で中止してしまったり (第 4 図)、菌柄は正常に伸びても菌傘が開かず (第 3 図) または半開 (第 2 図) という場合がしばしば見られた。上記のような不発育の原基や異常な子実体を一括してかりに“不開傘子実体”と呼ぶことにするが、もしこれの生ずる原因が遺伝的のものであるならば、その因子は標識因子として今後の研究実験に大いに役立つものと思ひ、この不開傘子実体について調べたところをここに報告する。

h 子実体からの 1 核菌糸体間の交配実験

前記の野生子実体 *h* から分離した単胞子培養のう

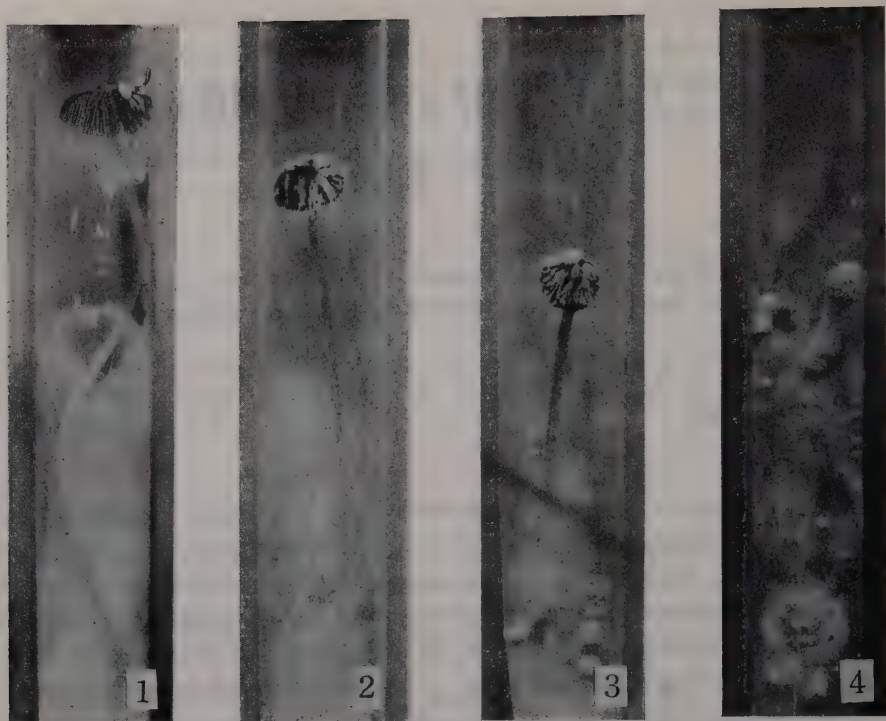
* 岡山大学理学部生物学教室 植物形態学業績 No. 80, 科学研究費 (課題番号 407130) による研究の一部。

** Department of Biology, Faculty of Science, Okayama University, Okayama, Japan. 岡山大学理学部生物学教室。

ち、任意の 20 を選び出し、相対する交配型のものどうしをあらゆる組み合わせで二つずつ試験管内培養基上に混植培養して、それぞれの子実体形成の状態を調べた。なお、全実験を通じ培養温度は 30° であり、培養基はペレイショ煎汁寒天を用いた。また、本菌の子実体の正常な発達には光を必要とするから、原基が現われ始めてからは定温器内に毎日、昼間だけ光を与え、子実体形成状態の決定は交配してから 15~20 日後に行なった。この実験は 3 回行なわれたが、その結果は第 1 表のごとくである。

各組み合わせ培養における 3 回の実験結果は必ずしも一致せず、不開傘子実体の生じた一つの組み合わせで、その不開傘の状態が相違していたり、また正常な子実体の生じた組み合わせにおいても原基が発育を中止するという場合があったが、これらはあとも述べるように各回における培養条件の間の差異によるものと一応見なせば、不開傘子実体は遺伝的に意味のある特定の組み合わせに生ずるものといえる。

すなわち、Zattler²⁾, Nobles³⁾, Macrae⁴⁾, Quintanilha and Balle⁵⁾, 武丸⁶⁾ らが述べているように、単相の 2 核をもつ帽菌類の 2 核菌糸体においても、複相の 1 核をもつ一般の生物の二倍体におけると同様に、メンデルの優劣の法則が認められるものであるが、いま正常な子実体形成の因子 *F* に対して劣性の不開傘子実体形成の因子 *f* を仮定し、野生子実体 *h* の因子型は *Ff* であり、供試単胞子培養のうち、交配型 *AB* の 10, 12, 13, *ab* の 6, *Ab* の 4, 9, 11, 17, *aB* の 19 は *F* 因子を、*AB* の



Figs. 1—4. Fruit-body formation of *Coprinus macrorhizus* form. *microsporus* on potato-sucrose agar slants in test-tubes. About natural size. Fig. 1, a normal fruit-body; Figs. 2—4, undeveloped fruit-bodies.

Table 1. Fruit-body formation on the dikaryons obtained by pairing of monosporous mycelia isolated from the wild fruit-body “h”. Explanation of signs in the following tables: F, normal fruit-bodies (Fig. 1); h, fruit-bodies with usual stipes and half-open pilei (Fig. 2); c, fruit-bodies with usual stipes and closed pilei (Fig. 3); r, undeveloped rudiments of fruit-bodies (Fig. 4). This experiment was completed for three times, and the results are indicated by three successive signs.

Monosporous mycelia		AB				
		7	8	10	12	13
ab	1	r c c	r r r	F F F	F F F	F r F
	2	r r c	r r c	F F F	F F F	r r F
	5	r r c	r r r	F F F	F F F	r F F
	6	F F F	r r F	F F F	r F F	r r F
	14	r r c	r r c	F F F	r r F	F r F
	20	r c c	c c c	F F F	F F F	F F F
Monosporous mycelia		Ab				
		3	4	9	11	17
aB	15	r r h	F F F	r F F	F F F	r r F
	18	r c c	F F F	r F F	r F F	r F F
	19	F F F	F F F	F F F	F F F	F F F
	23	r c r	r F F	F F F	F F F	r F F

7, 8, *ab* の 1, 2, 5, 14, 20, *Ab* の 3, *aB* の 15, 18, 23 は *f* 因子をもつものとすれば, 第1表の結果はうなずける.

この仮定を裏付けるため, 以下に述べるような実験を行なった.

検定交配実験

第1表の 1×10 は *abf* × *ABF* と仮定されたが, この交配によって生じた正常な子実体から 200 (Nos. 101~300) の単孢子培養を分離した. そのおのおのに, 第1表で *f* をもつと思われた単孢子培養の 7 (*ABf*), 1 (*abf*), 3 (*Abf*), 15 (*aBf*) をテストーとして交配して, 2核菌糸が出現するかどうか

によっておのおのの交配型を決めるとともに, 得られた2核菌糸体の子実体形成の状態を調べた. 子実体形成の試験は2回行ない, 2回の結果から不開傘か否かを決めがたかった少数のものについては, さらに行なった3回目の実験結果から, それを決定した.

この実験の結果の要約は第2表のごとくである. このデータから, まず *F* と *f* との分離を検討したのが第3表であるが, 1:1のメンデル分離を示し, 不開傘子実体の形成は単一の劣性因子 *f* によって支配されているものといえる. 次に *f* と不和合性因子 *A, B* との連鎖関係を分析したところ, 第4表に見られるように, *f* は *A, B* のいずれとも連鎖していないことがわかる.

Table 2. Fruit-body formation from pairing of four tester haplonts having *f*-factor with monosporous mycelia derived from the combination 1 (*abf*) × 10 (*ABF*) of Table 1. Tester haplonts *ABf*, *abf*, *Abf* and *aBf* are the monosporous mycelia Nos. 7, 1, 3 and 15 of Table 1, respectively.

Monosporous mycelia		Tester haplonts	Fruit-bodies	
Mating type	Number		Normal	Undeveloped
<i>AB</i>	52	<i>abf</i>	27	25
<i>ab</i>	45	<i>ABf</i>	18	27
<i>Ab</i>	53	<i>aBf</i>	23	30
<i>aB</i>	50	<i>Abf</i>	24	26
Total	200	—	92	108

Table 3. Segregation for *F, f; A, a; and B, b*.

	<i>F</i>	<i>f</i>	<i>A</i>	<i>a</i>	<i>B</i>	<i>b</i>
Number	92	108	105	95	102	98
Total	200		200		200	
χ^2 (for 1:1)	1.28		0.50		0.08	
P	0.30 ~ 0.20		0.50 ~ 0.30		0.80 ~ 0.70	

Table 4. Linkage data between *f*-factor and incompatibility-factors, *A* and *B*.

Loci	Parental combinations		Recombinations		Total	χ^2 (for 1:1)	P
<i>f, A</i>	<i>AF'</i>	<i>af</i>	<i>Af</i>	<i>aF'</i>			
	50	53	55	42			
	103		97		200	0.18	0.70 ~ 0.50
<i>f, B</i>	<i>BF'</i>	<i>bf</i>	<i>Bf</i>	<i>bF'</i>			
	51	57	51	41			
	108		92		200	1.28	0.30 ~ 0.20

この結果を再確認するために、前記 1×10 からの 単孢子培養のうちの No. 179 (abF') とテストの $7(ABf)$ との交配によって生じた正常な子実体より分離した単孢子培養 200 (Nos. 301~500) を用いて同じ実験を行なったが、第 5~7 表に示すように 同一の結果が得られた。木村¹⁾ は前に本菌の不和合性因子の A, B は、たがいに連鎖していないことを報告したが、第 2, 5 表のデータから再びこれについて検討したところ第 8 表に示すように前報告と同じ結果が得られた。

Table 5. Fruit-body formation from pairing of four tester haplonts having f -factor with monosporous mycelia derived from the combination $7(ABf) \times 179(abF')$. No. 179-haplont is one of the monosporous mycelia derived from the combination 1×10 .

Monosporous mycelia		Tester haplonts	Fruit-bodies	
Mating type	Number		Normal	Undeveloped
AB	41	abf	20	21
ab	62	ABf	30	32
Ab	49	aBf	21	28
aB	48	Abf	27	21
Total	200	—	98	102

Table 6. Segregation for $F, f; A, a;$ and B, b .

	F'	f	A	a	B	b
Number	98	102	90	110	89	111
Total	200		200		200	
χ^2 (for 1 : 1)	0.08		2.00		2.42	
P	0.80 ~ 0.70		0.20 ~ 0.10		0.20 ~ 0.10	

Table 7. Linkage data between f -factor and incompatibility-factors, A and B .

Loci	Parental combinations		Recombinations		Total	χ^2 (for 1 : 1)	P
f, A	Af	aF'	AF'	af			
	49	57	41	53			
	106		94		200	0.72	0.50~0.30
f, B	Bf	bF'	BF'	bf			
	42	51	47	60			
	93		107		200	0.98	0.50~0.30

Table 8. Linkage data between the two incompatibility-factors, A and B .

Combination	Parental combinations		Recombinations		Total	χ^2 (for 1 : 1)	P
	AB	ab	Ab	aB			
$1(ab) \times 10(AB)$	52	45	53	50			
	97		103		200	0.18	0.70~0.50
$7(AB) \times 179(ab)$	41	62	49	48			
	103		97		200	0.18	0.70~0.50

Ff 子実体の自殖実験

第1表も *Ff* 子実体の自殖実験の結果であるが、さらに前記の 7(*ABf*) \times 179(*abF*) よりの子実体から分離した *AB*, *ab*, *Ab*, *aB* の各交配型の単胞子培養のなかから、*F* のものと *f* のものとを四つずつ任意に選び出し、相対する交配型のものどうしの間

ですべての組み合わせ培養を行ない、おのおのの子実体形成の状態を調べた。この実験は1回行なっただけであるが、結果は第9表に示すように *F* \times *F*, *F* \times *f* のものには正常な子実体、*f* \times *f* のものには不開傘子実体が例外なく生じ、全く期待通りのものであったといことができる。

Table 9. Fruit-body formation from pairing of monosporous mycelia derived from the combination 7 \times 179.

Monosporous mycelia		<i>abF</i>				<i>abf</i>			
		397	406	418	421	312	336	342	369
<i>ABF</i>	305	F	F	F	F	F	F	F	F
	355	F	F	F	F	F	F	F	F
	396	F	F	F	F	F	F	F	F
	416	F	F	F	F	F	F	F	F
<i>ABf</i>	320	F	F	F	F	h	c	h	h
	327	F	F	F	F	h	h	h	h
	387	F	F	F	F	h	c	h	h
	395	F	F	F	F	r	h	r	r

Monosporous mycelia		<i>aBF</i>				<i>aBf</i>			
		410	419	420	455	314	321	337	383
<i>AbF</i>	354	F	F	F	F	F	F	F	F
	377	F	F	F	F	F	F	F	F
	392	F	F	F	F	F	F	F	F
	452	F	F	F	F	F	F	F	F
<i>Abf</i>	370	F	F	F	F	c	c	h	c
	382	F	F	F	F	c	r	h	c
	400	F	F	F	F	r	c	h	h
	407	F	F	F	F	c	c	r	c

***FF* と *ff* との交配実験**

第9表中の 396(*ABF*) \times 406(*abF*)ならびに 354(*abF*) \times 410(*aBF*) に生じた子実体から単胞子培養をそれぞれ40と35、分離した。これらは全部 *F* をもっているから、そのおのおのにテストの *ABf*, *abf*, *Abf*, *aBf* を交配すれば、その一つと和合して2核菌糸 *Ff* を生じ、この2核菌糸体は必ず正常な子実体を形成するはずである。このような期待のもとに行なわれたこの実験も、ただ1回の実験ですべての2核菌糸体に例外なく正常な子実体が生じた。

***ff* 子実体の自殖実験**

菌傘が開かないか、または半開の子実体においてもしばしば胞子が形成されるが、その量は正常な子実体に比べて、がいして少ない。第1表の 1(*abf*) \times 7(*ABf*) に生じた菌傘の開かない子実体から単胞子培養を分離したが、胞子の発芽は正常であった。得られた単胞子培養(Nos.501~520)をテストと交配して、それぞれの交配型を定めた後、四つの交配型群のおのおのから任意に三つずつを選び出し、相対する交配型のものどうしを交配して、その子実体形成の状態を調べた。3回行なったこの実験の結果は

第10表に示すように、ほとんど不開傘子実体ばかりで、まず期待に近いものであったが、506×508、506×514、508×509の組み合わせにおいて予期しなかった一見正常な子実体が生じたことがあった。

それで506×508に生じた正常子実体から分離した20の単胞子培養に*f*をもつテスターを交配して、生じた2核菌糸体の子実体形成を調べたところ、第11表に示す結果のように正常な子実体は全く現われなかった。このことから、*ff*の2核菌糸体においても、まれに一見正常な子実体が生ずることがあるが、これはそのときの培養条件による一時的のものであると考えられる。また第10表の502×507、その他の組み合わせで見られるように、実験の回に

よって不開傘子実体の状態が相違しているのも不明の外開条件の差によるものであろう。

第1表で正常子実体を作るべき*FF*または*Ff*の2核菌糸体に發育しない子実体原基が生じたことがあるのは、外開条件のほか供試した1核菌糸体の培養年令も原因していたと考えられる。それは本菌において単胞子分離後の1核菌糸体が時日を経るにつれて、それらの1核菌糸体どうしを組み合わせで作った2核菌糸体は生長速度が減じ、上記の不發育の原基が生じがちになるからである。第1表の供試1核菌糸体がこのような古いものであったのに対して、第9表の新しい1核菌糸体を用いた場合の実験では、*FF*および*Ff*の2核菌糸体がただ1回

Table 10. Fruit-body formation on the dikaryons obtained by pairing of monosporous mycelia from the undeveloped fruit-body, produced in the combination 1(*abf*)×7(*ABf*) of Table 1. This experiment was carried out three times, and the results are indicated by three successive signs.

Monosporous mycelia		<i>ab</i>		
		501	505	507
<i>AB</i>	502	r h h	r h h	r c h
	503	c r h	r c c	r c h
	504	r r h	c r r	c c h
Monosporous mycelia		<i>aB</i>		
		506	509	511
<i>Ab</i>	508	F r r	F r h	c r r
	512	r r h	r r h	r r r
	514	F F h	r r c	c c h

Table 11. Fruit-body formation from pairing of four tester haplonts having *f*-factor with monosporous mycelia isolated from the normal fruit-body, produced in the combination 506×508 of Table 10.

Monosporous mycelia		Tester haplonts	Fruit-bodies			
Mating type	Number		Normal	Undeveloped (r) (c) (h)		
<i>AB</i>	2	<i>abf</i>	0	1	1	0
<i>ab</i>	5	<i>ABf</i>	0	5	0	0
<i>Ab</i>	9	<i>aBf</i>	0	8	0	1
<i>aB</i>	4	<i>Abf</i>	0	4	0	0
Total	20	—	0	18	1	1

の実験で全部、正常な子実体を形成したことは、その証左とも考えられる。なお、 FF および Ff の 2 核菌糸体において、菌傘が半開または開かない子実体が生じた場合は、これまでの実験では認められなかった。

結 び

以上述べたようないろいろな交配実験の結果から不開傘子実体の形成は不和合性因子の A, B のいずれとも連鎖していない単一の劣性因子 f によるもの

であるといえる。そして、1) 半開または開かない菌傘をもつ子実体が生じたときは、その 2 核菌糸体は ff である、2) 発育しない原基が生じたときは、多くの場合、 ff であるが、培養年令の古い 1 核菌糸体どうしを組み合わせると 2 核菌糸体においては FF , または Ff のこともある、3) 正常な子実体が生じたときは、多くの場合、 FF または Ff であるが、まれに ff のこともある、などの条件をつけられ f 因子は標識因子として利用し得るものと思われる。

文 献

- 1) Kimura, K., Biol. Jour. Okayama Univ. 1 : 72 (1952).
- 2) Zattler, F., Zeit. Bot. 16 : 433 (1924).
- 3) Nobles, M. K., Mycologia 27 : 286 (1935).
- 4) Macrae, R., Nature 139 : 674 (1937).
- 5) Quintanilha, A., and Balle, S., Bol. Soc. Broter., Sér. 2, 14 : 17 (1940).
- 6) 武丸恒雄, 遺雑 32 : 286 (1957).
- 7) Kimura, K., Biol. Jour. Okayama Univ. 2 : 7 (1954).

Summary

Some of the dikaryons obtained by pairing of monosporous mycelia from the wild fruit-body "h" of *Coprinus macrorrhizus* form. *microsporus* (a tetrapolar fungus) produced undeveloped rudiments of fruit-bodies or abnormal fruit-bodies with usual stipes and closed or half-open pilei. This abnormality of fruit-body formation is a heritable character, being controlled by a single recessive factor, f . No linkage exists between the factor f and the incompatibility-factors, A and B .

Miscellaneous Notes

Hiroshi INOUE* and Naofumi KITAGAWA**: *Eremonotus* (Hepaticae), a New Addition to the Hepatic Flora of Japan

井上 浩*・北川尚史**: *Eremonotus* 属の日本における初発見

Received September 29, 1960

Eremonotus, a monotypic genus based on *E. myriocarpus*, has been known only in Europe, where this species seems to be very rare and is found in the subalpine or alpine zone (Müller 1954, Martenson 1955). Recently, this interesting hepatic was collected on two mountains in Japan, i.e. Mt. Hakkoda of Aomori Pref. (140° 50'E, 40° 40'N) and Mt. Goroyama of the Chichibu Mountains (138° 40'E, 35° 50'N). The junior author collected this species for the first time on Mt. Hakkoda (ca. 1500 m. alt.), on shaded, trickling or dripping volcanic rocks. No other hepatics were accompanied with *Eremonotus*, but adjacent to this, *Anthelia juratzukana* was found in a few quantity. The senior author found this species in Mr. D. Shimizu's collections made on the Chichibu Mountains. It was collected in wet crevices of granite, occurring with *Diplophyllum albicans*, at an elevation of about 2100 m.

Dr. H. Persson of Stockholm was kind enough to take trouble for us to verify our materials, informing us that the specimens were typical *Eremonotus myriocarpus*. Comparing the Japanese materials with those sent from Sweden by Dr. H. Persson, we could not find any prominent feature that might separate them from the Swedish plants. The plants from the Chichibu Mountains have some perianths while those from Mt. Hakkoda bear male inflorescences only.

The occurrence of this species in Japan is rather important from the phyto-geographical point of view. It has been reported neither from North America nor from the Himalayan regions. Its range of distribution is, therefore, quite discontinuous, leaving a wide gap between Europe and the Japanese Islands. The habitat condition of the Japanese plants seems to be nearly the same as that of the European ones. This species may be regarded as a relict of the preglacial flora. No locality other than the two, cited above, has been known to the authors, but the species is very difficult to recognize in the field and may have been overlooked elsewhere.

The authors are indebted to Dr. H. Persson of Stockholm for his verification of Japanese materials.

References

- 1) Müller, K., Die Lebermoose Europas, 3. Aufl., Lief. 5., Leipzig (1954). 2) Martenson, O., Bryophytes of the Tornetrask Area, Northern Swedish Lapland, Stockholm (1955).

* Botanical Institute, Faculty of Science, Tokyo University of Education, Tokyo; Hattori Botanical Laboratory, Miyazaki, Japan. 東京教育大学理学部植物学教室・服部植物研究所(兼).

** Department of Botany, Faculty of Science, Kyoto University, Kyoto, Japan. 京都大学理学部植物学教室.

Noboru HARA*: Apical Views of Vegetative Shoot Apex

原 襄*: 生長点の上方からの観察

Received November 22, 1960

The vegetative shoot apex commonly forms a dome. The uppermost section in a cross series of such a shoot apex contains only a few cells of the first tunica layer, as shown by Tepfer¹⁾. Therefore, we cannot trace cell lineage within this layer in serial cross sections as well as in serial longitudinal ones, although Newman²⁾ devised a method to observe apical views of a living shoot apex directly.

The shoot apex of *Daphne pseudo-mezereum* A. Gray is flat or slightly convex, and, if the sectioning is appropriate, the uppermost cross section contains a number of cells of the first tunica layer (Fig. 1 A), the second section containing centrally the cells of the second tunica layer with the peripheral cells which are in the first tunica layer (Fig. 1 B). If we pay attention only to the cells of the first tunica layer which are reconstructed in Fig. 2, we can observe centrally located cells at the centre and radial files of cells peripherally. The former cells show infrequent cell divisions and the latter frequent ones. The more peripheral the region, the greater the number of files.

In general, prior to the appearance of a leaf primordium cell divisions are predominantly tangential at a presumptive leaf site, and the shoot apex increases in width in this direction. Subsequently cells in the radial files, especially the central ones, divide radially rather actively.

As shown in Fig. 2, we can follow cell lineage within the first tunica layer in a more or less regular manner from the centrally located cells of the shoot apex to the highly protoplasmic superficial cells of the leaf primordium. The cell lineage is observed not only within the first tunica layer but within the second one (Fig. 1 B). Most, or at least a few, of the connecting cells in the lineage between the shoot apex and the leaf primordium are likely to be of use in passing morphogenetic substances from the apex to the leaf primordium. Even before the appearance of a leaf primordium, these correlations are distinct between the apex and a presumptive leaf site. Occasionally a few of the connecting cells resemble incipient procambium (Fig. 1 B). They are, however, not the precursors of the procambium. The latter arises later and is situated in the third to fifth layers in this species which has three to seven tunica layers.

With the subsequent elevation of the leaf primordium, the connecting cells become obscured by readjustment of the cells, although they form the residual meristem if the initial of an axillary bud arises. Therefore, it is appropriate to call the area of connecting cells the "evanescent apical cell connection".

The evanescent apical cell connection is of morphogenetic interest in considering the self-determining nature of the apical meristem, as discussed by Wardlaw³⁾ and others. Apical views of the shoot apex suggest various problems that still remain to be considered, regardless of many excellent studies of the shoot apex⁴⁾. More details concerning these problems will be reported later.

* Biological Institute, College of General Education, University of Tokyo, Tokyo, Japan.
東京大学教養学部生物学教室



Fig. 1. A, uppermost cross section of vegetative shoot apex of *Daphne pseudo-mezereum*. B, second section of same apex. A very young primordium scarcely showing elevation is at right. T1, first tunica layer; T2, second tunica layer; x, procambium-like cells of evanescent apical cell connection in second tunica layer. Section thickness is $10\ \mu$. $\times 400$.

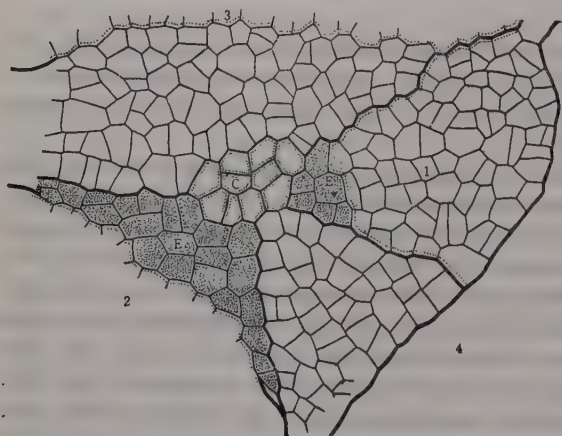


Fig. 2. Figure of first tunica layer which is reconstructed from figures of first tunica layer in Fig. 1 A and B, and reduced in one plane. C, centrally located cells; E, evanescent apical cell connection; 1, site of youngest leaf primordium; 2, 3, 4, successively older leaf primordia. $\times 400$.

References

- 1) Tepfer, S. S., Amer. Jour. Bot. **47**: 655 (1960).
- 2) Newman, I. V., Phytomorph. **6**: 1 (1956).
- 3) Wardlaw, C. W., Phylogeny and Morphogenesis, London (1952).
- 4) Gifford, E. M. Jr., Bot. Rev. **20**: 477 (1954).

Srinivasachary SAMPATH*: Notes on the Taxonomy of the Genus *Oryza*

S. Sampath: イネ属の分類学的知見

Received November 25, 1960

An enumeration of the species of *Oryza* as well as a key to the genus has been prepared by Chatterjee¹⁾. He recognizes 23 valid species from amongst the 30-40 specific names published previously. Since this publication, three new species have been described, bringing the total to 26. Though the genus is small, it is economically important and its taxonomy is of interest to workers tracing the interrelationships, origin, and evolution of cultivated rice. Kihara²⁾ has drawn attention to some probable errors in nomenclature. The present author has committed some errors in the past and the notes given below are intended to rectify them, and also to assist others in this field. This note is based on cytogenetical studies on *Oryza* collection at the Central Rice Research Institute, Cuttack, and observations on the *Oryza* species and hybrids being grown at the National Institute of Agricultural Sciences, Hiratsuka.

1) ***Oryza perennis*** Moench. Chatterjee¹⁾ includes under this specific name Asian, American, as well as African perennial wild rices. A group of African wild rices can be conveniently separated from *O. perennis* and renamed *O. barthii* A. Cheval. This species is characterized by erect habit, and strongly spreading rhizomes, as contrasted to the residual *O. perennis*, having weak rhizome development, and spreading, prostrate or floating habit. Both the species are adapted for outcrossing, but seed setting under selfing bags is partial in *O. perennis*, and nil in *O. barthii*. The failure to set seed on selfing, in *O. barthii* suggests self incompatibility, but, the observation needs confirmation. If self incompatibility exists, it is a distinctive feature, and a new specific name is justifiable. As far as is known, *O. barthii* and *O. perennis* have allopatric distribution, and hybridization between the two is difficult, and artificial cross pollinations have been hitherto unsuccessful.

2) ***O. cubensis***. The variety of wild rice occurring in Cuba must be included under *O. perennis* and Chatterjee¹⁾ does not record *O. cubensis* as a validly published name. The plants hitherto collected and grown have a semi-erect habit as contrasted to the floating or prostrate habit of Asian *O. perennis*, and since they are geographically isolated from the latter, can be given a varietal status, namely *O. perennis* var. *cubensis*.

3) ***O. paraguaensis***. There is no record of this specific name being published according to the rules of nomenclature. The South American plants given this specific name, are taxonomically identical with *O. alta* Swallen, and according to the rules this latter would be the valid name.

4) ***O. latifolia*** Desv. This name has been applied to two different species, one distributed in Tropical America and the other in Asia and possibly in Africa. The specific name *O. latifolia* is validly applicable to the American species which is a tetraploid ($2n=48$). The Asian species is diploid ($2n=24$) and is classifiable as *O. officinalis* Wall. In the standard book on Flora of British India, by Hooker, this species is listed as *O. latifolia* and the error has been copied by others. Chatterjee¹⁾ uses the character, ligule fringes, to discriminate between the two species, the tetraploid *O. latifolia* having fringed ligules, the diploid *O. officinalis* not having the fringes. This distinction is not absolute, because new observations have shown

* Colombo Plan Associate: National Institute of Agricultural Sciences, Hiratsuka, Japan.

the occurrence of fringes in new varieties of *O. officinalis* collected from Bombay State, from Thailand, and from China. In addition to this, a tetraploid species resembling *O. officinalis* has been recently collected from Kerala, India, by Krishnaswamy and Chandrasekaran³). This species has light fringing on ligules. It is desirable that a cytogenetic study of this species, along with new African collection at National Institute of Genetics, Mishima, be completed and species delimitations made. Till such a study is completed, it is desirable to use *O. latifolia* as the specific name for the American species, and *O. officinalis* for the Asian species.

5) *O. grandiglumis* (Doell) Prodoehl. Since the specific name implies increased size of glumes (sterile lemma), it has been wrongly applied to varieties of *O. sativa* and of *O. glaberrima* which had long glumes (e.g. Sampath and Rao⁴). In *O. ridleyi* also, the setaceous glumes are long, and this species has once been described as *O. grandiglumis*. The name is valid for an American species having the chromosome number $2n=48$. This species closely resembles *O. latifolia* Desv. and recent cytogenetical work at the National Institute of Agricultural Sciences, Hiratsuka, suggests that it is closely related to both *O. latifolia* and *O. alta*.

6) *O. meyeriana* (Zoll. et Mor.) and *O. granulata* Nees et Arn. These two species have been united by some taxonomists, and the Indian species is listed as *O. meyeriana* in some Floras. Both Chatterjee¹) and Backer⁵) have recorded that they are distinct and that the Indian species is *O. granulata*. This is diploid ($2n=24$) and suggestion by Sampath and Rao⁴) that it is tetraploid is wrong. *O. meyeriana* is an Indonesian species and *O. abromeitiana* is a synonym of this species. The chromosome number of this species is not established as the publications listing it as diploid do not show whether the genuine *O. meyeriana* was studied.

7) *O. punctata* Kotschy ex Steud. The taxonomy of this species requires further study. Sasaki⁶) has indicated that this African species is similar to *O. officinalis* of Asia, as well as to *O. latifolia* of America. Chatterjee¹) does not record the occurrence of *O. officinalis* in Africa. He distinguishes *O. punctata* from the latter in having longer spikelets (6–6.5 mm. long), longer awns (30–70 mm.) and longer ligules (4–6 mm.). Ligules are not fringed as they are in American species. A sample of seeds received from Belgian Congo were grown and studied. The Belgian taxonomists identify it as *O. schweinfurthiana*, a perennial type of *O. punctata*. This species had spikelets smaller than the limits for *O. punctata*, had the tetraploid chromosome number ($2n=48$) and was resembling *O. eichingeri*, another tetraploid species from East Africa. This collection is probably a new variety of *O. eichingeri*. It is also possible that *O. punctata* is a diploid species closely akin to *O. officinalis* and more variable than recognized by Chatterjee.

I am indebted to the Government for an award under Colombo Plan, which has enabled me to study botanical researches on rice in Japan. I am also indebted to Prof. T. Morinaga for facilities and encouragement.

References

- 1) Chatterjee, D., Ind. J. Agric. Sci. **18**: 185 (1948).
- 2) Kihara, H., Seiken Ziho **10**: 68 (1959).
- 3) Krishnaswamy, N., and Chandrasekaran, P., Sci. and Cult. **23**: 308 (1957).
- 4) Sampath, S., and Rao, M. B. V. N., Ind. J. Genet. Plt. Breed. **11**: 14 (1951).
- 5) Backer, C. A., Blumea Suppl. **3**: 44 (1946).
- 6) Sasaki, T., "On the distribution of *Oryza* species", (Japanese). Sakumotsu-gaku Ronshu, Kikkawa Kyoju Zaishoku 25 nen Kinenkai (1935).

本 会 記 事

役員異動

評議員選挙

会則第 8・9 条および付則第 3 第 2 条によつて、各支部ごとにおこなわれた評議員選挙の結果はつぎのようになりました。

北海道支部 (定員 2 名)

松浦 一, 山田幸男

東北支部 (定員 2 名)

長尾昌之, 吉田邦二

関東支部 (定員 10 名)

津山 尚, 林 孝三, 原 寛, 宝月欣二, 前川文夫, 三輪知雄, 門司正三, 八巻敏雄, 湯浅明, 亙理俊次

北陸支部 (定員 2 名)

柴田万年, 正宗敏敬

中部支部 (定員 2 名)

熊沢正夫, 田中 潔

近畿支部 (定員 5 名)

芦田譲治, 今村駿一郎, 神谷宣郎, 新家浪雄, 三木 茂

中国四国支部 (定員 4 名)

猪野俊平, 下斗米直昌, 堀川芳雄, 辰野誠次

九州支部 (定員 3 名)

千葉保胤, 野口 彰, 芳賀 恣

なお幹事, 編集委員にも一部異動がありました。新役員は表紙裏にまとめて掲載してあります。

支部通信

中部支部

第 63 回例会 (3 月 18 日, 名古屋大学理学部生物学教室において)

島村 環: 名古屋の植物学会・遺伝学会よもやま話, 16 ミリ映画映写

関東支部

関東支部大会 (4 月 7 日, 国立科学博物館において), 林 俊郎: 高等植物細胞の浮遊培養に関する研究, 中村輝子・柴岡弘郎・八巻敏雄: オークシンの細胞内分布, 柳沢新一: 桑樹に見出された B B 葉に対する考察, 山岸高旺: ホシミドロ科 (Zygnemataceae) の分類系, 草薙昭雄・田中信徳: Co^{60} - γ 線照射による *Luzula purpurea* の染色体異状について, 北見健彦: 佐渡ヶ島における *Monostroma* の一生態, 伊藤英子・根本祥子・大槻虎雄: 好塩性細菌の研究, 江森貫一: 荒川水系地区内のサクラソウ (*Primura*), 鈴木浩一: 紅色細菌における $2C^{14}$ -acetate の同化ならびにアミノ酸の合成, 井上 浩: ナンジャモンジャゴケに関する新知見, 岩崎尚彦: ジャジクモ科植物の生長点の分化と器官形成 *V. Nitella pseudoflabellata*, 広井敏男: 種々の相対照度下における群落の生長, 三輪知雄: 形態学と生理学, 前川文夫: ペルーの植物相

北海道支部

5 月例会 (5 月 13 日, 北大農学部において) 稲垣貫一: 道北原野の植生について, 細川定治: ヨーロッパのビート育種事情

なお, 中部支部長は熊沢正夫氏に, 北海道支部長は田川隆氏にそれぞれ決まりました。

本会会員岩淵初郎氏は 5 月 28 日死去されました。
ふかく哀悼の意を表します。

日 本 植 物 学 会

お 知 ら せ

4月号の赤紙、第26回大会（東京）のおしらせの中に、大会関係事項の連絡先が落ちていましたから、改めておしらせします。

連絡先： 東京都文京区本富士町、東京大学理学部植物学教室内、日本植物学会第26回大会準備委員会

10月14日（土）（植物学会大会会期中）午後6時から“酵母研究者の集まり”をひらきます。会食後、講演があります。ご来場をお待ちします（会費・会場未定）。

主催 酵 母 細 胞 研 究 会

まえにおしらせしましたように、本誌は今年度から、年間の総ページ数を1～2割ほどふやしました。それで、論文受理から誌上発表までの期間は、いままでより2～3カ月、短縮される見通しですから、このことをご承知のうえ、はやめにご寄稿ください。

日本ワックスマン財団より第5回研究助成金申請者の募集要項がきています。対象は微生物学の研究、および医学の研究で、申請受付締切は昭和36年9月15日です。申請用紙は学会事務にありますから、必要な方は請求してください。

日本植物学会会員名簿（昭和36年1月号所載）正誤表

頁	誤	正
4	◎朝比奈泰彦	◎*朝比奈泰彦
5	池上義信 新潟県立新潟南高校(新潟市上所島)	池上義信 新潟市上所島新潟南高校
5	浦口真左 港区芝功運町30 普連土学園(渋谷区伊達町21 柴沼方)	浦口直佐 港区芝三田功運町30 普連土学園
5	○大賀一郎	○*大賀一郎
6	○小倉 謙	○*小倉 謙
7	○小南 清	○*小南 清
8	○武田久吉	○*武田久吉
16	田川 隆	田川基二
16	西山市三 京大農食糧科学研究所	西山市三 京大農遺伝
18	○生駒義博	○*生駒義博
21	大橋 裕 九大薬生薬	大橋 裕 長崎市昭和町 863 長崎大薬生薬
22	本田康人	本多康人

Cracking of Bean Seeds Soaked in Water

by Mituo YAMAMOTO

Received October 25, 1960

Internal breaking of pea cotyledons due to uneven swelling was observed by Shull and Shull¹⁾. They explained an apparent increase in rate of water absorption as due to cavities formed by the breaking but did not relate it to germination. McCollum²⁾ described that a high incidence of cotyledonal cracking might be expected when susceptible varieties of snap beans were germinated in wet soil at low temperature after storage at low humidity, but no observation was made on the embryo axis. It is well known that bean seeds soaked in water decrease pronouncedly in germinative capacity^{3,4,5)}. In a previous paper⁶⁾, it was shown that well-dry bean seeds were impeded in germination by short-continued soaking. Temperature^{7,8)} and aeration⁹⁾ have considerable influences on soaking seeds. Literature which deals with the exact relationship between deleterious effect of soaking seeds and breakage of tissue is scanty.

The present study investigates whether or not breaking of seeds takes place during a soaking process, and if so, under what conditions, indicating that breaking of embryo axis leads to a decrease in germinative capacity of soaked seeds.

Materials and Methods

Seeds used were kidney bean (*Phaseolus vulgaris*) and soybean (*Glycine soja*) which had been stocked in the laboratory after harvest. Kidney bean was used for most of the experiments.

The seeds were divided into three lots, which were respectively stored at different humidities in order to change the moisture content before tests. One of the lots was placed over anhydrous calcium chloride for about two weeks, another lot was kept in a moist chamber for a few days, and the remaining lot was left as it was. Fifty seeds of each lot were used for the determination of the initial moisture content which was calculated on a dry weight after the seeds had been dried in a ventilated oven at 105°.

Tap water filtrated through ion exchange resin was used for the tests. Flasks of 300 ml. capacity containing 200 ml. of water were prepared, each of which received 25 seeds. Duplicate flasks, 50 seeds in all, were used for the same test. After soaking treatment, the seeds were carefully divided into cotyledons and embryo axis. Cotyledons were directly observed. The embryo axes were gradually dehydrated by ethyl alcohol and then transferred into xylol. Those which had become transparent by xylol were immediately observed or were cut into longitudinal sections with a microtome. Germinative capacity was determined by the seeds which had been placed in moist vermiculite at 20° to 30° for several days. The seeds which were placed on moist absorbent cotton without soaking in water were observed as a control. Observations were made only on the seeds which had imbibed.

* Department of Biology, Faculty of Liberal Arts and Science, Yamagata University, Yamagata, Japan.

Results

Well-dry seeds were soaked in water at 25° for 10 hours, and breaking of cotyledon and embryo axis was observed. The cotyledons of soaked seeds were easily separated from the embryo axis. One or more transverse fissures extended partially or completely across the cotyledon. Cotyledonal fissures of kidney bean and soybean are shown in Fig. 1-A and -B, respectively. Some cotyledons of the latter are disintegrated at the edge in addition to the transverse fissures.

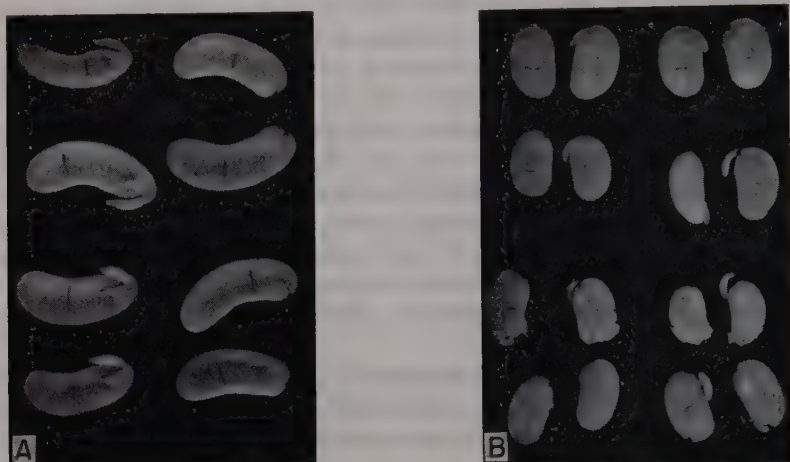


Fig. 1. Cracking of cotyledons. Seeds were soaked in water at 25° for 10 hours after storage at low humidity.

A) Kidney bean (initial moisture content: 3.5%).

B) Soybean (initial moisture content: 4.3%).

Many transverse fissures extend partially across the embryo axis, hypocotyl and radicle, as shown in Fig. 2-A. The fissures of embryo axis occur in the pith at first and then extend across the vascular elements to the cortex. The embryo axis which had been placed on moist absorbent cotton for 24 hours after the end of soaking was narrowed at the part with the fissure which extended to the cortex. This aspect is shown in Fig. 2-B. Embryo axis, the fissures of which exist only in the stele is quite normal in appearance (Fig. 2-A). Embryo axis of non-soaked seed without any fissures is shown in Fig. 2-C.

Experiments were performed to determine the relationship between condition of soaking and cracking of seeds. The three lots of kidney bean seeds which had been stored at different humidities had 3.5 (low), 12.5 (medium) and 62.0 (high) % initial moisture content, respectively. Cracking percentages were given by the respective numbers of cracked cotyledon and embryo axis per total seeds observed. Embryo axes were observed without cutting. Treatments similar to those described for observation of cracking were also made for germination test.

In Table 1 are shown the rates of cracking and germination obtained from the seeds which were placed on moist absorbent cotton without soaking in water at 5° or 25° for 24 hours.

As the seeds absorb water without soaking, most of them (above 94%) germinate vigorously and few fissures occur in the cotyledons and the embryo axes.

Cracking percentages of cotyledon and embryo axis, together with germination

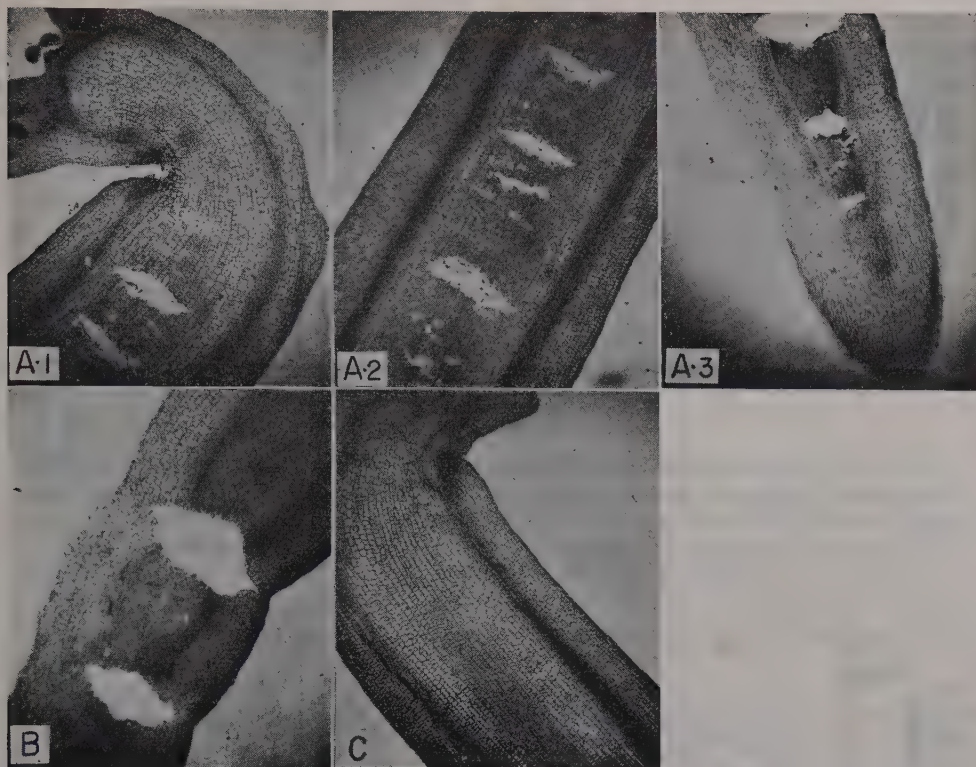


Fig. 2. Cracking of embryo axes of kidney bean (longitudinal section).

- A) Seed was soaked in water at 25° for 10 hours after storage at low humidity.
 B) Seed was placed on moist absorbent cotton at 25° for 24 hours after the treatment described above.
 C) Seed absorbed water without soaking in water.

Table 1. Cracking and germination of non-soaked seeds (kidney bean).

Temperature at which seeds absorbed water	Initial moisture content of seed %	Germination percentage	Cracking percentage	
			Cotyledon	Embryo axis
25°	3.5	96	8	2
	12.5	98	2	4
	62.0	98	2	0
5°	3.5	94	4	2
	12.5	96	2	0
	62.0	94	2	2

rates, are illustrated graphically in Fig. 3 for the seeds soaked in water at 25° for 4 to 24 hours.

The soaked seeds, except for those with the highest initial moisture content, show relatively high cracking percentage when the initial moisture content of the seeds is low. The seeds with 62.0% initial moisture content do not increase in cracking percentage by the soaking treatment. The cracking occurs within 4 hours after the start of soaking and is negatively correlated with the duration of soaking.

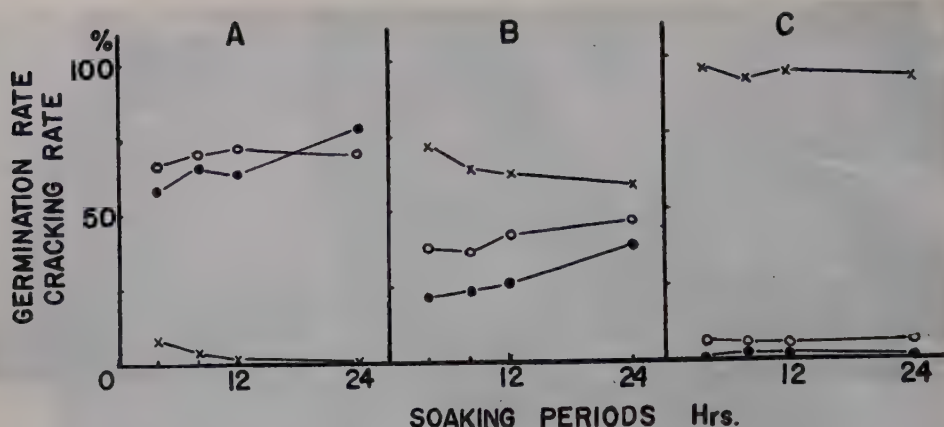


Fig. 3. Cracking percentages of cotyledon and embryo axis, and germination percentage (kidney bean). Seeds were soaked in water at 25°. Initial moisture content of the seeds: A 3.5%, B 12.5%, C 62.0%.

○ Cracking percentage of cotyledon. ● Cracking percentage of embryo axis.
× Germination percentage.

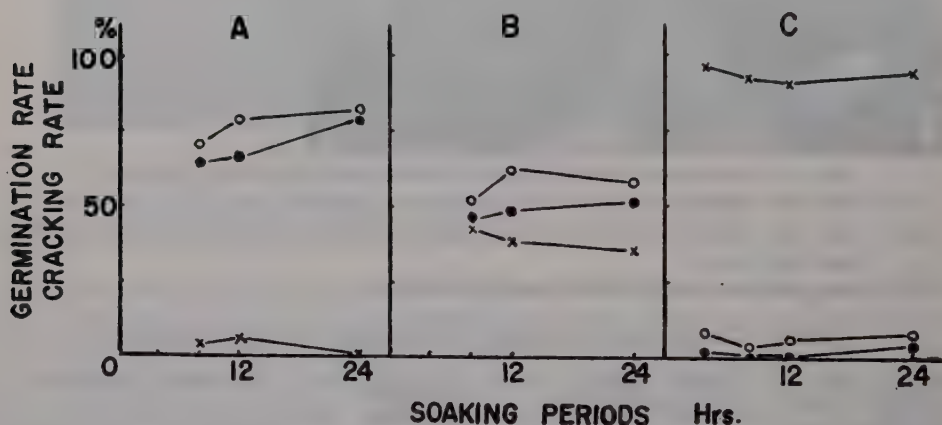


Fig. 4. Cracking percentages of cotyledon and embryo axis and germination percentage (kidney bean). Seeds were soaked in water at 5°. Indications as shown in Fig. 3.

The embryo axes of seeds which were soaked for 24 hours, however, increase slightly in cracking percentage.

The results with the seeds soaked in water at 5° are shown in Fig. 4. The seeds with 3.5 and 12.5% initial moisture content were soaked for not less than 8 hours, because there were many seeds which did not imbibe water during the shorter process of soaking.

The seeds with 3.5% initial moisture content, crack considerably in both cotyledon and embryo axis, during the soaking process, and the seeds with 62.0% initial moisture content have scarcely any fissures. As to the seeds with 3.5 and 62.0% initial moisture content, soaking at 5° brings the results similar to those obtained from soaking at 25°. The cracking percentage of the seeds with 12.5% initial moisture content is far higher at 5° than at 25°. The low temperature does not bring about the cracking by itself as shown in Table 1.

The next experiment was done to investigate the effect of aeration on cracking of seeds. Seeds were soaked in water through which air was slowly bubbled with an air pump during the soaking process at 25°. The results similar to those obtained from the experiment at the low temperature were shown in Fig. 5.

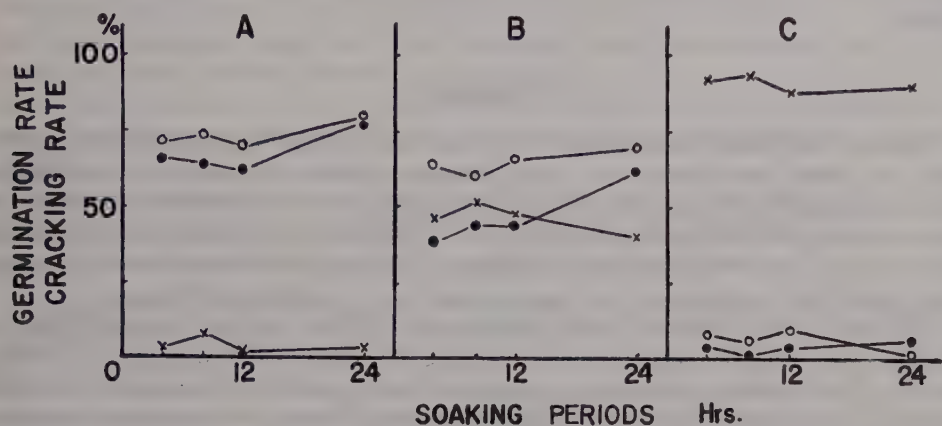


Fig. 5. Cracking percentages of cotyledon and embryo axis, and germination percentage (kidney bean). Seeds were soaked in water supplied with air at 25°. Indications as shown in Fig. 3.

The well-dry and the moist seeds are not influenced by soaking with aeration. The former crack and the latter do not. The cracking percentage of the seeds with 12.5% initial moisture content, however, is far higher in bubbled water than in non-bubbled water. Many fissures of the embryo axes soaked in bubbled water for 24 hours extended to the cortex.

The last experiment was made on the seeds soaked in a sucrose solution at 25° for 24 hours. Concentrations of the solution were 0.1, 0.3, 0.5 and 0.7 M. The results are shown in Fig. 6.

An increase in concentration of the solution reduces the cracking percentages

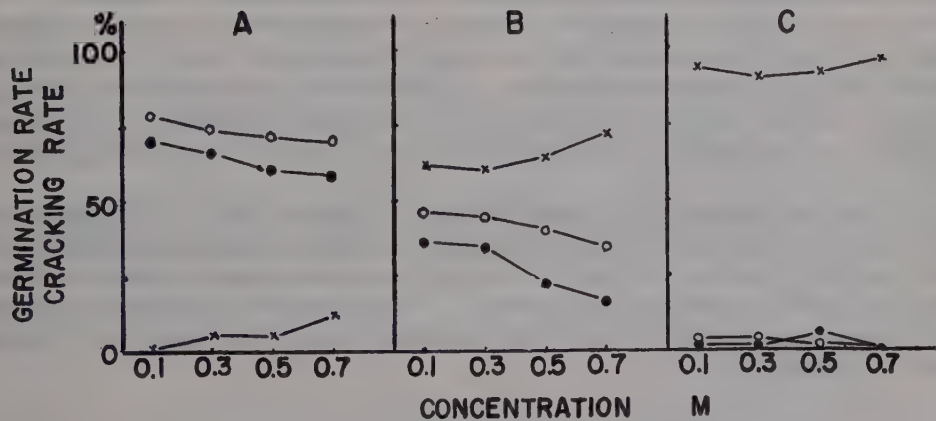


Fig. 6. Cracking percentages of cotyledon and embryo axis, and germination percentage (kidney bean). Seeds were soaked in sucrose solutions at 25° for 24 hours. Indications as shown in Fig. 3.

of the seeds with 3.5 and 12.5% initial moisture content. The seeds whose initial moisture content was increased before soaking are not affected by soaking in the solution.

In all the experiments, the cotyledons and the embryo axes are similarly affected and the germination is inhibited under the soaking conditions which enhance cracking of seeds.

Discussion

Some of the soaked seeds have many fissures in their cotyledons and embryo axes. As seeds were placed on moist absorbent cotton after soaking treatment or in water supplied with air, their embryo axes have one or more narrow parts with the fissures which have extended to the cortex. The fissure is spread with the growth of embryo axis, and the inhibition of growth in the part with the excessive cracking brings about the formation of the narrow part in the soaked embryo axis.

The seeds which imbibed water without soaking have but few fissures (Table 1). The cracking, therefore, does not occur during the storage but during the soaking process. The cracking percentage increases rapidly within 4 hours after the start of soaking, and later no further increase results. These facts apparently indicate that the cracking occurs during a short process of soaking. The increase in cracking percentage of the embryo axes soaked in water for 24 hours may be attributed to an easy observation on the fissures spread with the growth of embryo axes (Figs. 3, 5).

It is evident from the experimental results that the cracking is related with the initial moisture content of seeds. The well-dry seeds are seriously cracked by soaking in water under any conditions. An increase in initial moisture content of seeds prevents them from cracking caused by soaking, and no seeds with the high initial moisture content are cracked by soaking under any conditions. It is clear that the low and the high initial moisture content have greater influence on cracking than the external conditions during the soaking process have. Cracking of the seeds with the medium initial moisture content, however, is susceptible to influences of external conditions in soaking. This is due to the insufficient resistance brought with a slight increase in initial moisture content. The strong solution tends to prevent cracking of the soaked seeds, and the low temperature and the aeration to enhance it.

Low initial moisture contents⁸⁾ and aeration⁹⁾ expedite water absorption of seeds. Cracking of soaked seeds is associated with rapid absorption of water. Since a strong solution reduces water-uptake of seeds¹⁰⁾, it prevents the soaked seeds from cracking. Tissues of seed are unevenly swollen by rapid absorption of water and by unequal imbibition and elongation under low temperature, in which case cracking occurs in them.

Cracking occurs not only in cotyledon but in embryo axis. The soaking conditions which inhibit germination of soaked seeds are in agreement with the ones which augment cracking of them. It is found that embryo axes excised from cotyledons are able to continue the growth¹¹⁾. It is, therefore, impossible to suppose that the cracking of embryo axis is the immediate cause of the inhibition of germination by soaking.

Summary

To study on cracking of seeds with water soaking were used beans (kidney bean,

soybean) with different initial moisture contents.

Cracking occurs in both cotyledon and embryo axis in process of soaking. One or more transverse fissures extend partially or completely across the cotyledon or the embryo axis.

There is a direct relation between cracking and initial moisture content of seeds. Well-dry seeds produce many fissures by soaking under any conditions. An increase of initial moisture content of seeds prevents them from cracking, and well-moist seeds are rarely cracked by soaking under any condition. As to seeds with a medium initial moisture content, the cracking is enhanced by low temperature and aeration during the soaking process and reduced by a high concentration of sucrose solution.

Cracking of seeds occurs rapidly within 4 hours after the seeds were placed in water, and is negatively correlated with soaking of long durations.

Cracking of embryo axis directly impedes the germination.

I wish to express my thanks to Professor Dr. M. Kusa for the microphotographs appearing in this paper.

References

- 1) Shull, C. A., and Shull, S. O., Bot. Gaz. **93**: 376 (1932).
- 2) McCollum, J. P., Plant Physiol. **28**: 267 (1953).
- 3) Kidd, F., and West, C., Ann. App. Biol. **5**: 1 (1918).
- 4) Bailey, W. M., Bot. Gaz. **94**: 688 (1933).
- 5) Kisser, J., and Possnig, J., Beitr. Biol. Pflanzen **20**: 77 (1933).
- 6) Yamamoto, M., Bull. Yamagata Univ. Nat. Sci. **4**: 361 (1958).
- 7) Kidd, F., and West, C., New Phytol. **18**: 35 (1919).
- 8) Eyster, H. C., Amer. Jour. Bot. **23**: 691 (1936).
- 9) Barton, L. V., Contrib. Boyce Thompson Inst. **16**: 55 (1950).
- 10) Shull, C. A., Bot. Gaz. **62**: 1 (1916).
- 11) Yamamoto, M., Bull. Yamagata Univ. Nat. Sci. **4**: 479 (1959).

摘 要

山本光男： 浸水による豆の割れ目について

水に浸した豆の子葉および胚軸に割れ目ができる。おもにインゲンマメ (*Phaseolus vulgaris*) の種子を用いて、浸水によってできる種子の割れ目と、浸水前の種子含水量および浸水処理の状態との関係について実験を行なった。充分に乾燥した種子では、浸水によってつねに著しく、割れ目ができる。一方、あらかじめ吸湿した種子では、浸水中に割れ目ができない。風乾程度の含水量の種子は、低含水量の種子よりも割れかたが軽減されるが、その浸水処理の状態によって影響を受け、無通気 25°C 浸水の場合と比較して、通気あるいは低温の浸水によって割れ目が増大され、高濃度しよ糖溶液中では軽減される。この割れ目は浸水開始後4時間以内にでき、それ以上の長時間浸水にはあまり関係しない。しかし、胚軸はその成長とともに割れ目を拡大し、そのために切断されるようになる。これは長時間 25°C 通気の状態で浸水した種子、または短時間浸水したのち水から出して発芽させた種子の胚軸にみられる。割れ目は浸水初期の不均一な吸水によってできるものと思われる。種子が著しく割れる場合に発芽率は低下する。短時間浸水による発芽阻害は、浸水中に胚軸が割れることが大きく影響する。(山形大学文理学部生物学教室)

Ecological Studies of *Sasa* Communities

II. Seasonal Variations of Productive Structure and Annual Net Production in *Sasa* Communities*

by Yasuyuki OSHIMA**

Received December 2, 1960

In a previous paper¹⁾ it was clarified that the differences in standing crop of various *Sasa* communities were mainly caused by longevity of culms and rhizomes and no significant differences were found either in the leaf amount or in the light intensity in the communities with different standing crops. Elucidation of the relationships among these characters is awaiting a study which is concerned with the variation of productive structure in the course of community development.

Since Monsi and Saeki²⁾ had devised the stratifying clip method and established the method for growth analysis of plant community, studies on this line have intensively been carried out by many investigators³⁻¹⁰⁾. Some schemata also have been submitted concerning dry-matter reproduction, which determines the development of plant communities not only in quality but also in quantity^{8,11)}. The *Sasa* community, the growth of which has not yet been studied from this viewpoint, is surely of great interest.

In the present paper seasonal variation of productive structure mainly of closed *S. kurilensis* community at Mt. Waisuhorun (60 km. W from Sapporo, southern Hokkaido: Station D in a previous paper¹⁾) will be discussed on this line. Moreover, after the estimation of the annual dry weight increment, i.e. net production of the community, its ecological meaning on the development of the community will be discussed.

Variation with Time of Productive Structure

Sampling and measurements were made at the same stations and by the same methods as described in a previous paper¹⁾.

1. Total weight

In Table 1 are shown the seasonal variations of plant height, and of dry weights of leaves, culms, subterranean parts and the total in closed *Sasa kurilensis* communities at Mt. Waisuhorun and *S. nipponica* communities at Tomezuka (Station A, Mt. Kirigamine, Central Honshu). The former kept a fairly constant total dry weight of about 11 kg./m.² in every season of 1958-1960. This indicates that an annual equilibrium is established between the increment of living parts and the shedding of old parts, bringing about a steady state in the community. The same seems to be seen in general in closed *Sasa* communities under optimal conditions (see also the *S. nipponica* community in Table 1).

* A part of expense of this study was supported by a Grant-in-Aid from the Research Fund of the Ministry of Education.

** Department of Biology, Faculty of Science, Tokyo Metropolitan University, Setagaya, Tokyo, Japan.

Table 1. Plant height, number of culms, and weights of leaves, culms and subterranean parts of *Sasa kurilensis* and *S. nipponica* communities. Waisuhorun (Station D), 550 m. in altitude, southern Hokkaido; Tomezuka (Station A), 1700 m. in altitude, Central Honshu.

Station Species	Waisuhorun <i>Sasa kurilensis</i>						Tomezuka <i>Sasa nipponica</i>	
Sampling No.	D-4	D-5	D-7	D-2	D-3	D-6	A-1	A-3
Sampling date	Apr. 12 1959	Jun. 6 1959	Jul. 26 1960	Aug. 8 1959	Aug. 11 1958	Oct. 13 1959	Jul. 14 1957	Oct. 21 1957
Height (cm.)	—	297	328	325	325	330	95	102
No. of culms/m. ²	27	28	28	27	29	28	354	258
Leaves (g./m. ²)								
Fresh weight	840	815	895	875	850	820	665	620
Dry weight	410	465	465	455	445	420	275	260
Culms (g./m. ²)								
Fresh weight	12400	13060	12670	12450	13005	12225	1475	1390
Dry weight	7315	7635	7430	7280	7630	7090	605	510
Subterranean parts (g./m. ²)								
Fresh weight	11170	10650	9870	9340	9230	11300	1885	1910
Dry weight	3910	2720	3150	2985	2955	4170	820	845
Total (g./m. ²)								
Fresh weight	24410	24525	23435	22660	23085	24345	4025	3920
Dry weight	11635	12820	11055	10720	11030	11680	1700	1615
C/F ratio	27.4	24.4	22.8	22.5	23.8	26.8	5.2	5.2

2. Leaf weight and leaf area

The life duration of *Sasa* leaves was two whole years under favorable conditions.

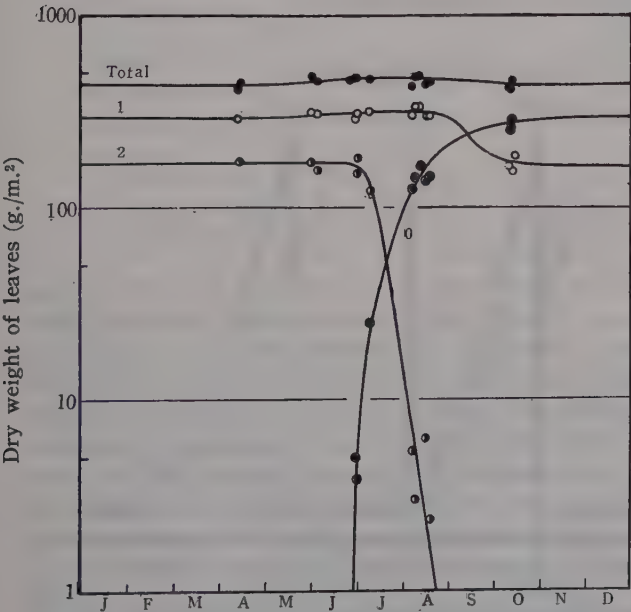


Fig. 1. Variation with time in dry weights of total, newly formed (0), one-year-old (1) and two-year-old (2) leaves of a *Sasa kurilensis* community at Mt. Waisuhorun, Hokkaido.

Fig. 1 shows the changes in dry weight of total leaves and of leaves of different ages in *S. kurilensis* community. The foliation of new leaves is observed in July–August, and they increase their dry weight till the end of October. In this season most of the 2-year-old leaves fall. A part of the one-year-old leaves situated in the lower part of foliage is shed in September. The current-year- and one-year-old leaves do not change their weight throughout the winter season under the protection of snow against low temperatures and severe dryness of the open air (cf. Fig. 2). As the foliation compensates the shedding of

old leaves, the total dry weight of the foliage is kept almost constant throughout the year; in this case it is ca. 450 g. dry weight/m.².

The ratios in dry weight of new leaves to the one-year-old ones in October in the communities of *S. kurilensis* at Mt. Waisuhorun, of *S. nikkoensis* at Mugikusa Pass of Mt. Yatsugatake and of *S. nipponica* at Mt. Kirigamine were 1.7, 1.6, and 1.8, respectively. There seems to be no significant difference. The total foliage weight of the *S. nipponica* community was also nearly constant throughout the year (Table 1). From these results it may be concluded that the time trend illustrated in Fig. 1 is a general feature of foliation and defoliation in closed *Sasa* communities of different species under

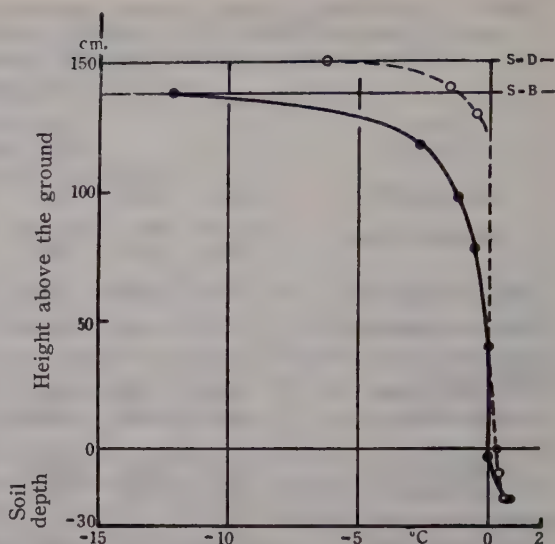


Fig. 2. Temperature distribution in the snow cover. S-D, snow surface at Station D (Mt. Waisuhorun, southern Hokkaido); S-B, snow surface at Station B (Mugikusa, Mt. Yatsugatake, Central Honshu).

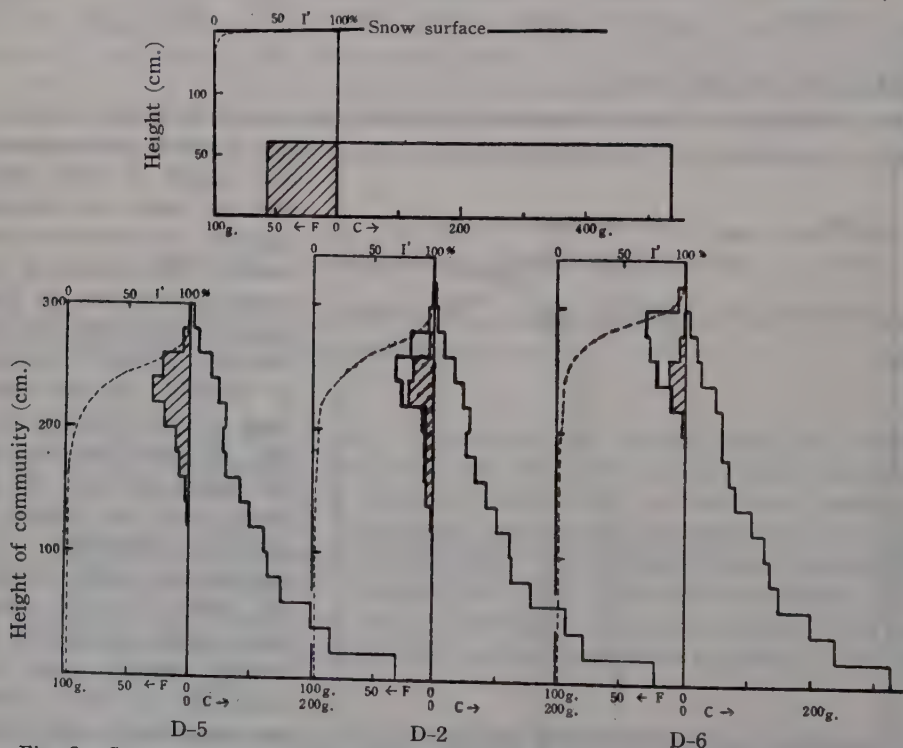


Fig. 3. Seasonal change of productive structure in the community of *Sasa kurilensis*. Photosynthetic system (F) consists of the laminae; non-photosynthetic system (C) of the leaf sheaths, branches, culms, etc; g. in fresh weight in (50 cm.²) land surface area. Black polygons, yellowish leaves; hatched, old leaves. D-4, April 12 (under snow); D-5, June 6 (after thawing); D-2, Aug. 8 (new leaves foliating and 2-year leaves defoliating); D-6, Oct. 13, 1959 (current-year leaves maturing, 1-year leaves remaining). See Table 1.

favorable conditions. Unfavorable conditions may make the time trend not always the same as that mentioned above. A detailed discussion will be presented in other paper.

As an example, seasonal variation of productive structure of *S. kurilensis* community is shown in Fig. 3 together with vertical distribution of relative light intensity. Throughout the growing season there are no marked changes.

With thawing of snow cover in late spring, a greater part of leaves and culms that have been bent down under the snow cover begin to recover their former productive structure by the strong elasticity of the culms. Some of culms, especially of aged culms having lost their elasticity, often remain as they bended. Consequently, the height of community is slightly lower from late spring to mid-summer than in autumn, and the leaves distribute in the former season vertically in a somewhat wider range than in the latter season. There is, however, no principal difference in the pattern of the productive structure in both seasons. At the end of June or the beginning of July—just after the vigorous elongation of new culms and branches—new leaves foliate at the uppermost layer of the community. Thus the new leaves decrease the intensity of light which the old leaves should receive. This makes most part of the shaded old leaves to defoliate with negative balance in dry matter economy. Hence, the lower limit of the foliage becomes higher and higher with the march of growing season. Thus, a productive structure of a closed *Sasa* community is maintained constant in quality as well as quantity throughout the growing period.

On the *Sasa kurilensis* community leaf area index, extinction coefficient and light transmissibility of a leaf were also determined (Table 2). These measures were

Table 2. Seasonal change of the leaf area index, the extinction coefficient and the relative light intensity (I_{min}) on the ground level of closed community of *Sasa kurilensis* at Mt. Waisuhorun (see Table 1 and Fig. 3).

Sampling No.	D-4	D-5	D-2	D-6
Sampling date	Apr. 12	Jun. 6	Aug. 8	Oct. 13
Leaf area index	5.1	5.1	5.2	5.1
Extinction coefficient	—	0.75	0.73	0.74
I_{min} (%)				
observed range	0.01	1.1-7.1	1.0-5.4	0.9-7.3
mean	0.01	2.1	2.6	3.1
calculated	—	2.2	2.2	2.3

nearly constant throughout the year; leaf area index, ca. 5.1, extinction coefficient, 0.75, and light transmissibility, 0.09. Saeki¹²⁾ has presented the following equation concerning the light intensity (I) received actually by the leaves in a plant community, $I/I_0 = K \exp(-KF)/(1-m)$, where m is light transmissibility of a leaf; the others are already mentioned¹⁾. Using the corresponding values obtained in the *Sasa kurilensis* community, the calculated light intensities at 2 m. high were 4.8, 2.8 and 1.9% in June, August and October, respectively.

Such high utilization of light, and structural characters of the community—e.g. quick recovery of productive structure after thawing, maintenance of evergreen leaves—seem to be responsible for its large annual production.

3. Light intensities on the floor of the *Sasa* community

In a previous paper¹⁾ the author reported that the relative light intensity at the ground level falls in a narrow range of deviations in all *Sasa* communities studied.

For the analysis of stability of community, it needs detailed studies on the light intensities in the community throughout the year, as the light intensities can decide the fate of seedlings growing in the community. The mean relative light intensities and their deviation range measured in various seasons were 2-3%, and 1-7%, respectively. The light intensity calculated with the values of K and F in Table 2 was at the ground level about 2% during the growing season.

Light intensity at the ground level decreases rapidly with accumulation of snow. For instance, the relative light intensity was ca. 5% at the depth of 5 cm., and only 0.1% at the depth of 20 cm. The matter production of *Sasa* community under snow must be negligible because of low light intensity and low temperature.

4. Seasonal change in weight of culm and subterranean parts

No significant seasonal changes of culm weight are observed in a community of a certain species (Table 1); e.g. deviation of 7.1-7.6 kg. dry weight/m.² was observed in the *S. kurilensis* community at Mt. Waisuhoron, and of 0.5-0.6 kg., in the *S. nipponica* community at Mt. Kirigamine. There exists an annual equilibrium between the increment and the withering of culms.

On the other hand, there is a clear seasonal change in the weight of subterranean parts,—it begins to decrease in the late spring and then rapidly decreases to reach a minimum in the summer, but recovers rapidly in the autumn. In the *S. kurilensis* community, the minimum of 3.0 kg. dry weight/m.² was observed in the middle of August, and the maximum of 4.2 kg. in October (see Table 1 and Fig. 4). Rhizomes occupy about 3/4 of the dry weight of subterranean parts.

The greater part of annual increment on the non-photosynthetic system is mainly caused by new formation of the system, because main culms, branches and rhizomes of over one

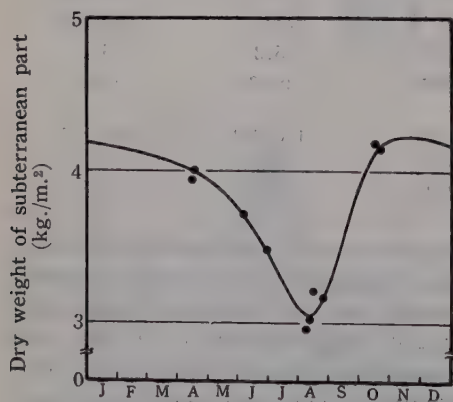


Fig. 4. Variation with time of total dry weight of subterranean part in the *Sasa kurilensis* community.

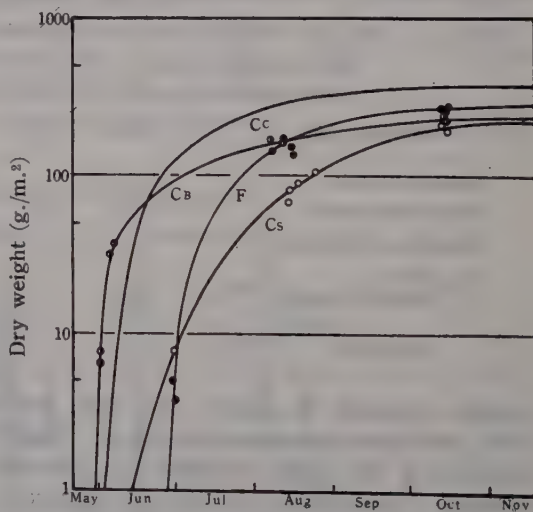


Fig. 5. Growth curves in dry weights of new leaves, culms, branches and rhizomes of the *Sasa kurilensis* community. F, Cc, Cb and Cs at the curve indicate leaves, culms, branches and rhizomes, respectively.

year hardly increase in height and thickness in the following years. The slight increase in weight of these organs in younger stage is caused by the thickening of cell membranes.

The weight of newly formed branches and rhizomes was estimated directly using a quadrat of 2×2 m.² (Fig. 5). The annual increment of mean dry weight of new

culms was estimated indirectly. For the estimation, a large survey area on 25 m.² was prepared, in which the date of sprouting, the daily elongation rate in each developing stage of young shoots, the number of sprouting culms, the diameter at the third internode above the ground level, and the dry weight of culms of various

height were measured. The mean diameter was 1.5 cm., and the density, 2.7 culms/m.². The young shoots sprouted out late in May, 1959 or early in June, 1960. The mean elongation rate of young shoots in 1959 was shown in Table 3. On the basis of these results, the growth curve in dry weight of a *Sasa kurilensis* community could be illustrated in Fig. 5.

The height and weight of the new culms and branches increased rapidly in June and July. Early in July a maximum daily elongation of 14.8 cm. was observed in a culm of 165 cm. high. Even after attainment of the maximum height, increase in the weight continued till the end of autumn. In the early stage of

growth, new rhizomes showed the smaller rates of elongation and weight growth than did the culms and branches. They continued their growth with the same rate until the mid-autumn. By the end of the growth period, the increases in dry weight of newly formed culms, branches and rhizomes were respectively 390, 245 and 230 g./m.², and that of newly formed roots was about 80 g./m.²; the latter was assessed with the ratio in dry weight of root to rhizomes which was mentioned before.

The subterranean parts and older culms lose their dry weight in June and July with rapid growth of younger culms and branches, but afterwards they recover their weight rapidly, as mentioned before and in Table 4. A similar change of reserved

Table 4. Mean dry weight of 20 dominant 3-year-old culms, which were in average 1.6 cm. in diameter.

Sampling date	Apr. 25, 1960	Jun. 4, 1960	Aug. 18, 1959	Oct. 12, 1959
Main culm (g.)	237	231	192	243
New leaves (g.)	—	—	18	31
New branches (g.)	—	0.4	17	24
Old leaves (g.)	43	46	32	17
Old branches (g.)	105	100	98	82

starch in culms and rhizomes was recently reported by Uéda *et al.*¹³⁾ in *Pleioblastus pubescens*. Here come into question the translocation of reserved substances into newly formed tissues, the amount of respiration loss in reserve organs, and the translocation of photosynthate into culms and subterranean parts⁸⁾.

The ratio of conversion from reserved substances into active organs, i.e. Midori-kawa's "economic ratio"⁸⁾ or Monsi's "transformation factor"¹¹⁾ was about 0.5–0.6 in *Aconitum*⁸⁾, potato⁸⁾, and *Helianthus tuberosus*¹⁰⁾. The ratio in *Sasa kurilensis* calculated from the data in Figs. 4 and 5 is also about 0.5. The rapid growth of culms and branches in early summer takes place under sufficient supply of reserved substances from well developed rhizomes and culms of older ages. On the other hand, some

suppressed culms and branches must be forced to die in the shade of foliation of new leaves. Consequently, it is probable that the substances necessary for the growth of new culms, rhizomes and roots in the early growing stage are almost covered by the stored material in the well-developed rhizomes, and the substances for the new branches mainly by the storage in the older culms. These characteristics of *Sasa* which are advantageous to the maintenance of the constant productive structure may contribute to high productivity, and consequently to the dominancy in competition with other plants with different pattern of matter reproduction^{8,11}).

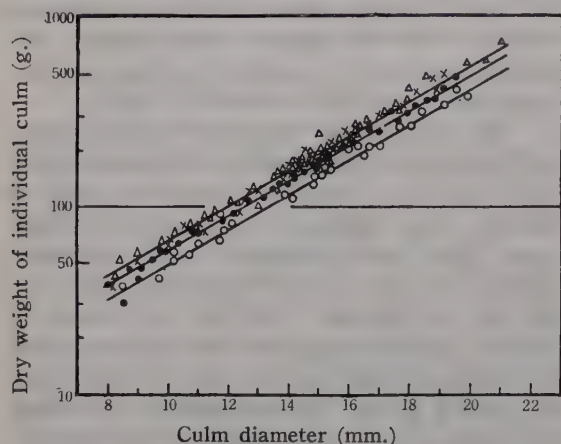


Fig. 6. Relation between the diameter of the third internode above the ground level and the dry weight of culm of *Sasa kurilensis* in October. Open circle, solid, triangle and cross marks indicate the culms newly formed, one year old, two years old and more than three years old of age, respectively.

increase in diameter. By using these linear relations between the dry weight and the diameter, which were easy to measure directly in the stand, the rate of annual increment of older main culm was calculated for each age (Table 5). It was assumed that branches and rhizomes increased every year at the similar rate to that at which culms did.

Annual Net Production of the *Sasa kurilensis* Community

The amount of net production (P_n) is determined by the balance between photosynthetic gain and respiratory loss during a given period, i.e. $P_n = F(a-r) - Cr_0$, where F , a , and r indicate the total amount of photosynthetic system and its photosynthetic and respiratory rate, while C and r_0 , the total amount and respiratory rate of non-photosynthetic system (culms, branches, rhizomes and roots^{5,6,10}). The production is distributed into both systems, F and C , according to distribution ratio, which can be influenced by environmental conditions and determines the weight of both systems after the period. Annual net production in a plant community is the sum of annual dry weight increment of both systems. In the case of a perennial community, even if the annual dry weight growth is zero—in the *S. kurilensis* community at Mt. Waisuhorun, the total dry weight was rather constant for three years, 1958–1960 (see also Table 1)—the annual net production is given as the sum of total dry weight of newly formed parts and the annual dry matter increment in the older parts.

Logarithm of dry weight of main culms of the same age increases linearly with increase in diameter measured at the third internode above the ground level, except for the suppressed ones (Fig. 6). The weight of the main culm increases with increasing age, in spite of no marked

Table 5. Ratios in dry weight of main culms to those of the previous year. Measured in *Sasa kurilensis* at Mt. Waisuhorun, on October 15, 1959.

1st year/current year	1.20
2nd year/1st year	1.11
3rd year/2nd year	1.04
4th year/3rd year	1.00

The total dry weights of newly formed leaves (ΔF), branches (ΔC_B), culms (ΔC_C), rhizomes (ΔC_S), and roots (ΔC_W) were estimated from the data already illustrated in Figs. 1 and 5. Annual increment in dry weight of older culms ($\Delta C_C'$) was calculated from the growth of culms in Table 5, and those of branches ($\Delta C_B'$), rhizomes ($\Delta C_S'$) and roots ($\Delta C_W'$) were calculated on the assumption that these organs increase in every year with the same rate as in $\Delta C_C'$. Moreover, the amount of branches withered within a year had to be added to correct the underestimation, because a part of branches lived only for two years. In the case of leaves at the end of autumn, the annual dry weight increment ($\Delta F'$) of old leaves was estimated as a balance between the increment of 13% of new leaves and the loss by leaf shedding (Table 6). Yellowish leaves decrease their dry weight to 87% of green ones of the

Table 6. Dry weight of newly formed leaves, culms, branches, rhizomes and roots in communities, of *Sasa kurilensis* and *S. nikkoensis* and dry weight increments of older parts in the former. In g./m.²/year.

	<i>S. kurilensis</i>		<i>S. nikkoensis</i>
	New	Old	New
Leaves	290	20	220
Culms	390	150	610
Branches	245	75	
Rhizomes	230	90	260
Roots	80	30	90
Total	1235	365	1180

Annual net production 1600 g./m.²

same age, and dead culms, to 88%. If dead branches, rhizomes and roots also decrease their dry weights to 88% of the initial ones as dead culms do and the substances which disappeared from these organs are translocated into the living parts before withering, the annual net production may amount to 1425 g./m.².

The dry weight increments of each organ were also determined in the *Sasa nikkoensis* community in a subalpine grassland near Mugikusa Pass in October, 1960. The values obtained are almost the same as measured in the *S. kurilensis* community (see Table 6). The annual dry weight increments in each organ of different ages in *S. nikkoensis*, though not determined, seem to be similar to those in *S. kurilensis*, because the annual increases in total dry weight, i.e. the sum total of annual dry weight increments, of both communities slightly differ from each other (see Table 7).

Table 7. Dry weight of non-photosynthetic system of aerial part (branches and main culms) of each age in closed communities of *Sasa kurilensis* and *S. nikkoensis*. Observed in Oct.

Community	Dry weight of each age (g./m. ²)			
	Current	1	2	≥3
<i>S. kurilensis</i> -comm.	645	700	730	5225
<i>S. nikkoensis</i> -comm.	610	585	660	390

Here it may be concluded that the amounts of annual net production in closed *Sasa* communities of different species can be similar to each other under optimal environ-

mental conditions. However, this by no means implies that the annual gross production in closed *Sasa* communities is also nearly the same amount regardless of the differences of species. For example, annual total respiration loss appears to be larger in *S. kurilensis* community than in *S. nikkoensis* community, because the former has a larger non-photosynthetic system (see Table 5 and Fig. 3 in a previous paper¹⁾) with the same temperature coefficient of respiration.

The annual net production of ca. 1.6 kg./m.² in *S. kurilensis* community (Table 6), is larger than that of a 30-year-old birch forest (1.2 kg./m.²; unpublished) standing near the sampling station, and nearly the same or somewhat larger than those of deciduous or coniferous forests determined in Europe¹⁴⁻¹⁷⁾ and Japan^{4,9)}, where the climate is similar to or slightly cooler than that of Mt. Waisuhorun. In herb communities, annual net production of 1.55 kg./m.² was estimated in *Aconitum altherbosa* at Mt. Hakkoda⁸⁾. Although very few natural communities in Japan have so far been reported on their annual net production, their maximum standing crops are known of some grassland and high moor communities in cool temperature or subarctic zones^{2, 18-20)}. Provided that a perennial herb community can represent its annual net production roughly by its maximum standing crop, it is evident that the productivity of the *Sasa* communities is nearly the same as or somewhat higher than those of herb communities with exception of extremely high productivity of *Cirsium nipponicum*¹⁸⁾, *Miscanthus sinensis*²⁾ and *Phragmites communis*¹⁹⁾ communities.

Time trends of the daily dry weight increments of newly formed leaves, culms, branches and rhizomes, and of a stand in the *Sasa kurilensis* community are shown in Fig. 7. The increment of the stand reaches a maximum of 11 g./m.²/day late in

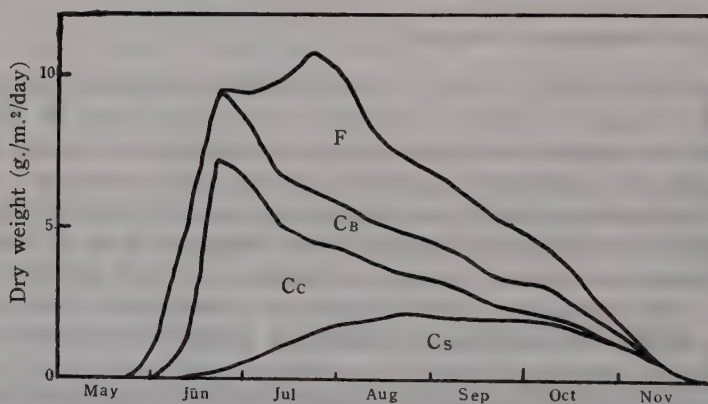


Fig. 7. Seasonal variation of calculated daily dry weight increment in newly formed leaves (F), culms (Cc), branches (CB) and rhizomes (Cs) of the *Sasa kurilensis* community.

July: this value is obviously far small in comparison with the maximum daily net production of ca. 20 g./m.² in *Aconitum* community⁸⁾ and ca. 30 g./m.² in an artificial community of *Helianthus tuberosus*¹⁰⁾. The growth period of *Aconitum* and *Helianthus* is limited to 4.5 and 5 months, respectively, while that of *S. kurilensis* at Mt. Waisuhorun is 6 whole months. The fact that in the *Sasa* community a large amount of annual net production is probably due to continuation of high productivity throughout the growing season, which is closely related to the specific structural characters.

Summary

The characteristics of productive structure and its seasonal changes were analyzed in the closed *Sasa kurilensis* community at Mt. Waisuhorun, 60 km. W from Sapporo, Hokkaido, and in some other *Sasa* communities. Annual net production was also estimated in those *Sasa* communities.

1. The *S. kurilensis* community showed a nearly constant standing crop of 11 kg. dry weight/m.² throughout the year. This means that the amount of newly produced part in each organ was almost equal to that of withered part.

2. With the foliation of new leaves at the end of June, the 2-year-old leaves generally defoliated to keep the amount of whole leaves at a constant level of ca. 0.85 kg. wet weight/m.², or ca. 5 in leaf area index. Productive structure can rapidly recover after thawing from the inactive state in winter by strong elasticity of culms. These facts must play an important role for the large matter production and the large standing crop of the community.

3. Relative light intensities under the *Sasa* community fall in a narrow range of 1-7%, the mean being 2-3%. Such low light intensities can prevent the invasion of other plant species.

4. Dry weight of culms showed nearly a constant value, 7.5 kg./m.², throughout the year. The subterranean parts, however, varied in dry weight, i.e. the maximum of 4.2 kg. in early November and minimum of 3.0 kg. in mid-August. Culms, branches and rhizomes newly formed during the growing season were 390, 245 and 230 g. dry weight/m.², respectively. A linear relationship was observed between the diameter and the logarithm of dry weight of culms.

5. The decrease of dry weight in culms and rhizomes, probably of reserved starch was recognized in late spring-early summer when the rapid growth occurred, and then increased till late autumn. Transformation factor from reserved substances into active organs appeared to be 0.5.

6. *Sasa kurilensis* community kept its daily net production at fairly high level throughout the growing season, and a maximum of newly formed increment was about 11 g./m.²/day. The annual net production was estimated to be 1.6 kg./m.²/year.

7. Productive structure and its seasonal variation were studied also in *S. nipponica*- and *S. nikkoensis*-communities. The latter could achieve almost the same amount of net production as that of the *S. kurilensis* community, in spite of the smaller standing crop.

The author wishes to express his gratitude to Prof. K. Hogetsu of Tokyo Metropolitan University and Prof. M. Monsi of the University of Tokyo for their guidance and suggestion under which this work was carried out. Thanks for help of various kinds are also due to Mr. I. Matsuoka, Mr. O. Tonouchi, Dr. B. Midorikawa and Mr. M. Kimura.

References

- 1) Oshima, Y., Bot. Mag. Tokyo **74**: 199 (1961).
- 2) Monsi, M., and Saeki, T., Jap. J. Bot. **14**: 22 (1953).
- 3) Sato, T., Nakamura, K., and Senda, M., Bull. Tokyo Univ. Forests, **48**: 65 (1955).
- 4) —, Kunugi, R., and Kumekawa, A., ibid. **52**: 33 (1956).
- 5) Iwaki, H., Jap. J. Bot. **16**: 210 (1958).
- 6) —, ibid. **17**: 120 (1959).
- 7) Kuroiwa, S., Bot. Mag. Tokyo **72**: 413 (1959).
- 8) Midorikawa, B., Ecol. Rev. **15**: 83 (1959).
- 9) Kuroiwa, S., Bot. Mag. Tokyo **73**: 133, 165 (1960).
- 10) Hogetsu, K., Oshima, Y., Midorikawa, B., Sakamoto, M., Tezuka, Y., Mototani, I., and Kimura, M., Jap. J. Bot. **17**: 278 (1960).
- 11) Monsi, M., Bot. Mag. Tokyo **73**: 81 (1960).
- 12) Saeki, T.,

ibid. **73**: 55 (1960). 13) Ueda, K., and Uchimura E., Bull Kyoto Univ. Forests **27**: 112 (1958). 14) Müller, D., Planta **16**: 1 (1932). 15) Möller, C. M., Det Forst. Forsøsv. Denmark **17**: 1 (1944). 16) Möller, C. M., Müller, D., and Nielsen, J., ibid. **21**: 253, 273, 327 (1954). 17) Ovington, J. D., Ann. Bot. N. S. **21**: 287 (1957). 18) Hogetsu, K., Ichimura, S., Hori, S., Oshima, Y., Kasanaga, H., Ono, H., and Takada, K., Sci. Res. Ozegahara Moor, Tokyo: 313 (1954). 19) Kurasawa, H., and Sakamoto, M., Misc. Rep. Res. Inst. Natur. Resour. **40**: 81 (1956). 20) —, and —, ibid. **43-44**: 30 (1957).

摘 要

大島康行: ササ群落の生態学的研究.

II. ササ群落の生産構造の季節的变化と年純生産量

すでに報告したと同じ北海道ワイスホルン岳のチシマザサ, および場所と種を異にする二, 三のササのよく発達した純群落地で, 生産構造の季節的变化と年純生産量を調べた.

単位面積当たりの現存量, 葉量, 桿 (主桿+枝) 量は一年の間ほぼ一定の値を維持しており, ササ群落の生産構造も積雪期をのぞいてほとんど変化がなかった. すなわちチシマザサ群落では年間を通じ現存量は乾量で約 11 kg., 葉量約 0.45 g., 桿量約 7.3 kg./m.² 葉面積指数は約 5 であった. これはおもに (1) 桿の強い弾力性のために, 冬期, 雪下に常緑の葉が低温と乾燥から保護されており, 雪解けとともにただちに桿が立ち前年とはほぼ同じ生産構造に回復するため, (2) 7月上旬群落上方に新生葉が展開するに伴って群落内部の光条件が低下し, 下部にある二年生葉の大部分と一年生葉の一部は枯死し, これら枯死葉をつけた桿や枝もまた枯死し, 新生量と枯死量とがほぼ均合しているためであることが明らかになった. また個々の主桿の直径と乾量の対数との間に直線関係がみられた. ササ群落内部の相対照度は積雪期をのぞいてほぼ一定で, 平均 2~3%, 変動の中も 1~7% という低い値を示した.

一方, ササの新生器官が急速に生長する 6 月下旬から 8 月上旬まで地下部の重さは急速に減少し, チシマザサ群落では 8 月中旬最小値 3.0 kg./m.² を示した. その後地下部の重さは増大し, チシマザサは 11 月上旬最大値の 4.0 kg./m.² に達した. これはおもに地下部に含まれる貯蔵物質質量の変化によっており, 桿についても同様の傾向がみられ, これら貯蔵物質の新生器官への転形率は約 0.5 であった.

葉, 桿, 地下部の乾量の増分から純生産量を求めた. チシマザサ群落では積雪期をのぞく約 6 カ月の間比較的高い増分が維持され, 新生器官の 1 日当たりの増大の最大は約 11 g./m.² であった. また年間の新生葉, 主桿, 枝の増分はそれぞれ 230 g., 390 g., 245 g./m.² であり, 年純生産量は 1.6 kg./m.² といふかなり高い値が得られた. チシマザサ群落より現存量の小さいニッコウザサ群落でも年純生産量はほぼ同様の値を示した. (東京都立大学理学部生物学教室)

Physiological Studies on the Fertilization in *Lythrum salicaria* Linn.

I. Presence of Pollen-germination Inhibitors in the Ovary

by Tamio TATEBE*

Received December 15, 1960

The physiological mechanism causing self-incompatibility of the homomorphic (homostylic) plants is considered to be due to some inhibitory substances contained in the pistil. On the other hand, very little is known as yet regarding the physiological mechanism controlling incompatibility of the heteromorphic (heterostylic) plants. The problem of heterostylism in *Lythrum salicaria* has been investigated by a number of workers since the time of Charles Darwin¹). From the pollen-tube behavior of *Lythrum salicaria*, Schoch-Bodmer²) supposed that the mechanism causing incompatibility should be due to some inhibitory substances contained in the pistil. However, the physiological evidence for her hypothesis is not clearly established as yet. Esser³) described that in *Lythrum salicaria* the addition of stigmatic, stylar, or pollen extract to a culture medium showed neither an alteration in the percentage of pollen germination nor an acceleration or inhibition of the pollen-tube growth. In the present experiments, the addition of crushed stylar tissue to a standard medium showed a slight inhibition of pollen germination. But the addition of crushed ovarian tissue induced a strong inhibition of pollen germination. The results obtained and their significance will be described in the following pages.

Material and Methods

The material used, *Lythrum salicaria* Linn. var. *roseum superbum* Hort., was raised from commercial seed. As is well known, *Lythrum salicaria* is a tristylous plant, viz., (1) long-styled plants having long style, mid stamens (mid pollen), and short stamens (small pollen), (2) mid-styled plants having mid style, long stamens (large pollen), and short stamens (small pollen), and (3) short-styled plants having short style, long stamens (large pollen), and mid stamens (mid pollen). Generally speaking, the pollinations between stigmas and anthers at the same level are compatible (legitimate unions), but those between stigmas and anthers at different levels are incompatible (illegitimate unions). After Bodmer⁴) and Esser³), a standard medium composed of 1 per cent agar-agar and 25 per cent sucrose (about pH 5.4) was used in the present tests for pollen germination. To prepare the test media, the fresh pistillary organs were crushed in an agate mortar, into which a small amount of the standard medium was poured and stirred thoroughly, and then the mixture was rapidly poured into a petri-dish (60 mm. in diameter). The number of the pistillary organs which were added to the standard medium was as follows. (1) Stigma and style: 3 in long-styled flower, 4 in mid-styled flower, and 10 in short-styled flower. (2) Ovary: 4 in each flower type. After the pollen was brushed on the medium, the petri-dish was transferred into a moist chamber (a zinc box containing a little water at the bottom). The experiments in 1958 and 1959 were carried out at room

* Kamiizumi-cho, Ibaraki-shi, Osaka-fu, Japan.

temperature (about 22°). Bodmer⁴) reported that the optimum temperature for pollen germination in *Lythrum* is 25–27°. Following her data the experiments in 1960 were carried out in a thermostat regulated at 27°.

Results

The pollen of *Lythrum salicaria* germinated well and developed long pollen-tubes on the standard medium. Table 1 gives the average percentage of pollen germination from experiments carried out repeatedly, the length of pollen-tubes being incapable of measuring, because the pollen-tubes were long and entangled with one another. The addition of crushed stigmas and styles to the standard medium showed no

Table 1. Pollen germination on standard medium.

Pollen from	% germination (No. of pollen grains observed)		
	1958	1959	1960
IM	56.2 (978)	59.2 (4120)	71.1 (5198)
IS	81.2 (739)	72.5 (5011)	88.2 (5005)
mL	76.1 (813)	65.3 (5820)	60.5 (6275)
mS	86.9 (756)	76.4 (4871)	89.1 (4798)
sL	68.3 (909)	57.4 (5610)	67.5 (4936)
sM	60.1 (859)	70.0 (4242)	82.4 (5746)

l: large-pollen, m: mid-pollen, s: small-pollen, L: long-styled flower, M: mid-styled flower, and S: short-styled flower. Combined symbol IM indicates large-pollen from mid-styled flower, and similarly so forth.

marked effect on pollen germination, as was reported by Esser³). On the contrary, the pollen germination was inhibited strongly on the medium into which crushed entire pistils were added. Further, the addition of crushed ovaries to the standard medium inhibited very strongly the pollen germination in the cases corresponding to illegitimate unions, suggesting the existence of some inhibitory substance or substances in the ovary. Unexpectedly, the pollen germination in the experiments corresponding to legitimate unions was also inhibited by adding crushed ovaries to the standard medium, as was confirmed by repeating the experiments.

The results presented in Table 2 show, from the first line to the fourth, (1) the germination tests of small pollen from long-styled flowers (sL) on the standard medium, (2) those on the medium added with crushed stigmas and styles from long-styled flowers, (L), and (3) those on the medium added with crushed ovaries from L, the latter two corresponding to illegitimate unions. The fourth (4) shows the results of germination tests of large pollen from mid-styled flowers (IM) on the medium added with crushed ovaries from L, which corresponds to legitimate unions. The same classification of the germination tests as just mentioned above was followed in the subsequent categories. The data given in Table 2 indicate the averages of the values obtained by the experiments repeated 10 times in each tests. Upon a careful inspection of the data obtained in 1959 and 1960, it will be noted that in illegitimate combinations the addition of crushed stigmas and styles to the standard medium is slightly inhibitory to pollen germination, but that of crushed ovaries induces a strong inhibition. In legitimate combinations, the addition of ovaries causes a medium inhibition to pollen germination contrary to expectation.

Table 2. The effect on pollen germination of adding the crushed pistillary organs to the standard medium.

Medium	Pollen from	% Germination (No. of pollen grains obs.)		% Pollen bursting*	
		1959	1960	1959	1960
Standard medium	sL	57.4 (5610)	67.5 (4936)	4.0	1.3
+stigmas and styles from L	sL	55.9 (7192)	77.0 (9692)	42.3	28.0
+ovaries from L	sL	3.0 (5324)	15.7 (8841)	100.0	98.5
+ovaries from L	IM	6.5 (5541)	55.9 (9253)	97.2	93.0
Standard medium	mL	65.3 (5820)	60.5 (6275)	0.3	1.2
+stigmas and styles from L	mL	64.1 (7521)	61.1 (9473)	27.8	17.3
+ovaries from L	mL	21.2 (7452)	1.6 (8632)	96.2	100.0
+ovaries from L	IS	36.4 (7771)	51.4 (10133)	93.3	98.6
Standard medium	sM	70.0 (4242)	82.4 (5746)	2.4	0.7
+stigmas and styles from M	sM	63.8 (6822)	64.5 (8372)	30.1	7.0
+ovaries from M	sM	21.6 (7654)	17.2 (11723)	98.8	99.0
+ovaries from M	mL	20.0 (7592)	20.4 (8721)	97.4	98.3
Standard medium	IM	59.2 (4120)	71.1 (5198)	9.8	4.2
+stigmas and styles from M	IM	51.2 (7352)	76.9 (10543)	53.5	7.0
+ovaries from M	IM	4.1 (7331)	15.8 (7841)	100.0	99.2
+ovaries from M	mS	48.8 (7045)	43.3 (8642)	100.0	100.0
Standard medium	IS	72.5 (5011)	88.2 (5005)	3.9	3.5
+stigmas and styles from S	IS	53.5 (6561)	67.9 (9091)	72.1	6.4
+ovaries from S	IS	29.0 (5235)	5.5 (9233)	100.0	100.0
+ovaries from S	sL	38.8 (4493)	54.2 (11012)	100.0	93.6
Standard medium	mS	76.4 (4871)	89.1 (4798)	0.3	1.1
+stigmas and styles from S	mS	59.6 (6843)	68.1 (9253)	58.8	1.0
+ovaries from S	mS	33.6 (7325)	11.2 (12521)	100.0	100.0
+ovaries from S	sM	22.0 (4761)	49.1 (9013)	100.0	98.9

* Bursting pollen in total pollen germinated.

Discussion

Previously, Jost⁵⁾ remarked briefly the immunological explanation for the phenomenon of self-incompatibility in the homomorphic plants. At his time, however, little attention was paid to it. Afterwards, East^{6), 7)} suggested that a mutual reaction, analogous to the immunity reaction, occurs between stylar tissue and pollen-tubes. And he⁶⁾ proposed that "if we assume that the secretions of a pollen-tube bearing a given gene, say S_1 , act as antigens against the stylar tissue bearing the gene S_1 ; if we further assume that the stylar tissue in which the S_1 is present forms antibodies against such a pollen-tube and thus inhibits its growth; then all requirements are satisfied", that is, immunity theory. This theory has recently been proved in parts serologically by Lewis⁸⁾, and biochemically utilizing radio-active tracers by Linkens^{9, 10, 11)}. According to Lewis⁸⁾, however, in *Oenothera organensis* antibodies are

formed in the style without stimulation by pollen antigens, as was proved by double pollination. For, the first pollination with incompatible pollen does not enhance the inhibition to the second incompatible pollination. In *Raphanus sativus*, Kroh¹²⁾ proved the diffusion of inhibitory substance from the stigma into a drop of gelatine. Consequently, the immunity theory has not yet been established convincingly.

Only little is known as yet regarding the physiological mechanism controlling incompatibility of the heteromorphic plants. Zollikofer¹³⁾ reported that the grafting of style or the shortening of style in *Primula* enhanced fertility in illegitimate unions, suggesting the presence of some inhibitory substances. This finding is closely similar to those known in self-incompatible homomorphic plants, e.g., *Brassica* and *Raphanus* (Sears¹⁴⁾, Tatebe^{15, 16)} and Kroh¹²⁾). In two homomorphic plants, *Oenothera organensis* and *Prunus avium*, Lewis¹⁷⁾ demonstrated that the rate of pollen-tube growth in compatible styles increases with a rise in temperature, while in incompatible styles pollen-tube growth is retarded by high temperature. The same fact was also manifested in two heterostyled species, *Primula obconica* and *P. sinensis* (cf. Lewis¹⁷⁾). According to Schoch-Bodmer²⁾, the pollen-tube behavior of *Lythrum salicaria* suggests a mutual reaction, chemical but not physical, between pollen-tube and conducting tissue. As is well known, bud-fertility is noted in numerous self-incompatible homomorphic plants. Esser³⁾ has recently described bud-fertility in *Lythrum salicaria*. This is the first case of bud-fertility reported in the heterostyled plants. According to Esser³⁾, and Esser and Straub¹⁸⁾, the mechanisms of incompatibility in hetero- and in homomorphic plants closely resemble each other, supporting in part the immunity theory.

Genetically speaking, there are two types of self-incompatibility in the homomorphic plants, that is, gametophytic and sporophytic. Both types are governed by multiple alleles at a single locus, *S*, and rarely by alleles at two different loci. In the gametophytic system, pollen behavior is determined gametophytically by the *S* allele contained in pollen grain itself. While in the sporophytic system, pollen behavior is determined sporophytically; in other words it is controlled by the paternal genotype. In the gametophytic self-incompatible homomorphic plants, autopolyploids produced by colchicine become often self-compatible, e.g., *Antirrhinum* and *Petunia*¹⁹⁾. On the other hand, in the sporophytic self-incompatible homomorphic plants, autopolyploids do not change their incompatibility relationships, e.g., *Brassica* and *Raphanus*^{19, 20)}. With respect to the heteromorphic plants with sporophytic system, Esser³⁾ has failed in *Lythrum* and *Fagopyrum* to remove the incompatibility by the chromosome doubling.

Some fifty years ago Correns²¹⁾ found, in self-incompatible *Cardamine pratensis*, stigmas from an individual squashed in a drop of cultural medium to inhibit the germination of its pollen. His experiment thus suggests the presence of inhibitory substances in the stigma. This inhibitory effect was induced with stigmas not only from the same individual, but also from other ones. Self-incompatibility in *Petunia violacea* was investigated by observing pollen and pollen-tube behavior on the cultural media which contained stigmatic secrete, crushed pistil, or crushed ovary, and by some other experimental procedures, e.g., the grafting of style and the shortening of style (Yasuda²²⁾). On the basis of the data obtained by a series of tests, he proposed that some inhibitory substances are produced in the placenta which diffuse upward to the style, inhibit self- and accelerate cross-fertilization. On the other hand, Straub²³⁾ reported in *Petunia hybrida* the addition of crushed style or stylar extract to a medium to inhibit not only the pollen-tube growth of the pollen of the individual,

from which the style was excised, but also that of the pollen from other individuals. According to him, therefore, the pollen-tube growth in self- and cross-pollination can not be distinguished by the germination test on the medium containing crushed style or stylar extract. Tatebe²⁴) has also pointed out in *Raphanus sativus* that the addition of crushed pistils to a medium checks not only self-pollen, but also cross-pollen.

As has been mentioned above, in *Lythrum salicaria* the addition of crushed ovaries to the medium inhibits the pollen germination in legitimate as well as in illegitimate combination. This finding in *Lythrum salicaria* resembles those found in self-incompatible homomorphic plants, *Cardamine pratensis*, *Petunia hybrida*, and *Raphanus sativus*. Consequently, it seems likely that the present experiments in *Lythrum salicaria* support the view of immunity theory, as in the case of the self-incompatible homomorphic plants. However, further experiments are needed to substantiate the hypothesis put forward here.

Summary

The pollen of tristylous *Lythrum salicaria* germinates well on a medium composed of 1 per cent agar-agar and 25 per cent sucrose. The addition of crushed ovarian tissues to the medium inhibits the pollen germination in legitimate as well as in illegitimate combination. As is known in some self-incompatible homostylous plants, the addition of crushed pistils to a medium inhibits the pollen germination and pollen-tube growth in cross- as well as in self-combination. Basing on this similarity, it is likely that the incompatibility in tristylous *Lythrum salicaria* should be due to some inhibitory substances.

References

- 1) Darwin, C., The Different Forms of Flowers on Plants of the same Species, John Murray, London (1877).
- 2) Schoch-Bodmer, H., Arch. Julius Klaus-Stiftg. **20** (Ergänzungsband): 403 (1945).
- 3) Esser, K., Zeits. f. indukt. Abst. u. Vererbungslehre **85**: 28 (1953).
- 4) Bodmer, H., Flora (Jena) N. F. **22**: 306 (1927).
- 5) Jost, L., Bot. Ztg. **65**: 77 (1907).
- 6) East, E. M., Self Sterility, Bibliogr. Genetica **5**: 331 (1929).
- 7) —, Proc. Nat. Acad. Sci. (Washington) **20**: 364 (1934).
- 8) Lewis, D., Proc. Roy. Soc. (London) Ser. B, Biol. Sci. **140**: 127 (1952).
- 9) Linskens, H., Zeits. f. Bot. **43**: 1 (1955).
- 10) —, Ber. dtsch. Bot. Ges. **71**: 3 (1958).
- 11) —, ibid. **72**: 84 (1959).
- 12) Kroh, M., Zeits. f. indukt. Abst. u. Vererbungslehre **83**: 365 (1956).
- 13) Zollikofer, Cl., Planta **16**: 763 (1932).
- 14) Sears, E. R., Genetics **22**: 130 (1937).
- 15) Tatebe, T., Journ. Hort. Assoc. Japan. **10**: 62 (1939).
- 16) —, ibid. **24**: 168 (1955).
- 17) Lewis, D., Proc. Roy. Soc. (London) Ser. B, Biol. Sci. **131**: 13 (1942).
- 18) Esser, K., and Straub, J., Biol. Zentralb. **73**: 449 (1954).
- 19) Lewis, L., Advances in Genetics **6**: 235 (1954).
- 20) Beteman, A. J., Heredity **9**: 53 (1955).
- 21) Correns, C., Biol. Zentralb. **33**: 389 (1913).
- 22) Yasuda, S., Bull. Imp. Coll. Agric. a. Forest. Morioka (Japan) **20**: 1 (1934).
- 23) Straub, J., Zeits. f. Naturforsch. **1**: 287 (1946).
- 24) Tatebe, T., Journ. Hort. Assoc. Japan. **16**: 106 (1947).

摘 要

建部民雄： エゾミソハギの受精に関する生理学的研究

I. 子房内に含まれる花粉発芽抑制物質

エゾミソハギは周知のように、個体によってそれぞれ3種類の異なる花形をもつ長短花柱植物である。その花粉は人工培地(25% ショ糖, 1% 寒天)上でよく発芽し花粉管を伸ばす。この培地に子房組織汁を加えて花粉の発芽試験を行なうと、花粉の発芽は、不和合の組合せにおいて強い抑制を、また和合組合せにおいても中度の抑制をうけた。すでに自家不和合植物においては、培地にめしべの組織汁を加えて花粉の発芽試験を行なうと、自家ならびに他家の花粉の発芽や花粉管の伸長が抑制される場合が知られている。

従来自家不和合性の機構は、めしべの中の抑制物質によるものと考えている学者が多いが、長短花柱植物の不和合性の機構は、今のところまだほとんど不明の状態である。ところが近年この現象も、自家不和合性の場合に似た抑制物質の作用によるのではないかと考える学者がしだいに多くなってきた。今回の実験成績もエゾミソハギの不和合性は子房内に含まれる抑制物質によることを暗示している。(大阪府茨木市上泉町)

Studies on the Phosphorylase Action in Plants

I. Histochemical Investigation of Phosphorylase in Growing Leaf Epidermis

by Isao KATO*

Received December 19, 1960

Yin *et al.*^{1,2)}, using a histochemical method, have found strong action of phosphorylase in stomatal guard cells of tobacco and broad bean leaves, and suggested that the enzyme plays a part in stomatal movements by changing osmotic value of the cells.

Although in leaves of a variety of plants, phosphorylase action likely appears solely in guard cells, in *Phytolacca americana* and *Datura tatula*, it is detectable also in other epidermal cells other than guard cells (Kato and Fukuda³⁾). On the other hand, Konagamitsu and Ono⁴⁾ have observed the phosphorylase action in epidermal and mesophyll cells of young leaves, but only in guard cells of mature leaves of liliaceous plants.

The purpose of the present study is to disclose more definitely the change in distribution of phosphorylase in growing leaf cells so as to gain some clue to the role which may be played by the enzyme in plant cells.

Material and Methods

The leaf epidermal tissues of *Tradescantia reflexa*, *Datura tatula* and *Vigna catiangu* were used as the experimental materials. Most experiments were made with *Tradescantia*, because of the ease of stripping off epidermal tissues from the leaf blade.

The slices of stripped epidermis were incubated for about 24 hrs. in a reaction mixture which was kept at 25° in a thermostat. Then the materials were stained with iodine solution, and were examined under a microscope to see whether starch is formed or not. The control samples were incubated in the reaction mixture without G-1-P (α -D-glucose-1-phosphate).

The reaction mixture consisted of 1% G-1-P solution, adjusted to pH 6.0 with acetate buffer, and a few drops of toluene which was known to increase the permeability of cells to G-1-P (Stocking⁵⁾).

Results

Experiment in *Tradescantia reflexa* Along the axis of a leaf blade (3~5 cm. in length) of *Tradescantia*, a zonal distribution of the age is present. Thus the blade was arbitrarily separated into four parts, namely a, b, c and d (Fig. 1). Epidermal tissues excised from b, c and d contained stomatal cells at different matura-

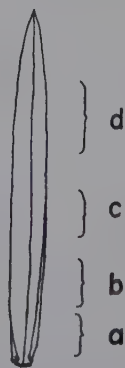


Fig. 1. Schematic diagram of a young leaf (3~5 cm. in length) of *Tradescantia reflexa* showing four maturation stages (a, b, c and d) examined.

* Biological Institute, College of General Education, Hiroshima University, Hiroshima, Japan.

tion stages and those from part a were consisted solely of meristematic cells, as shown in Fig. 2.

(A)~(D) in Fig. 2 show starch formation in epidermal tissues treated with G-1-P solution. Each protodermal cell in the basal part (part a in Fig. 1) containing starch

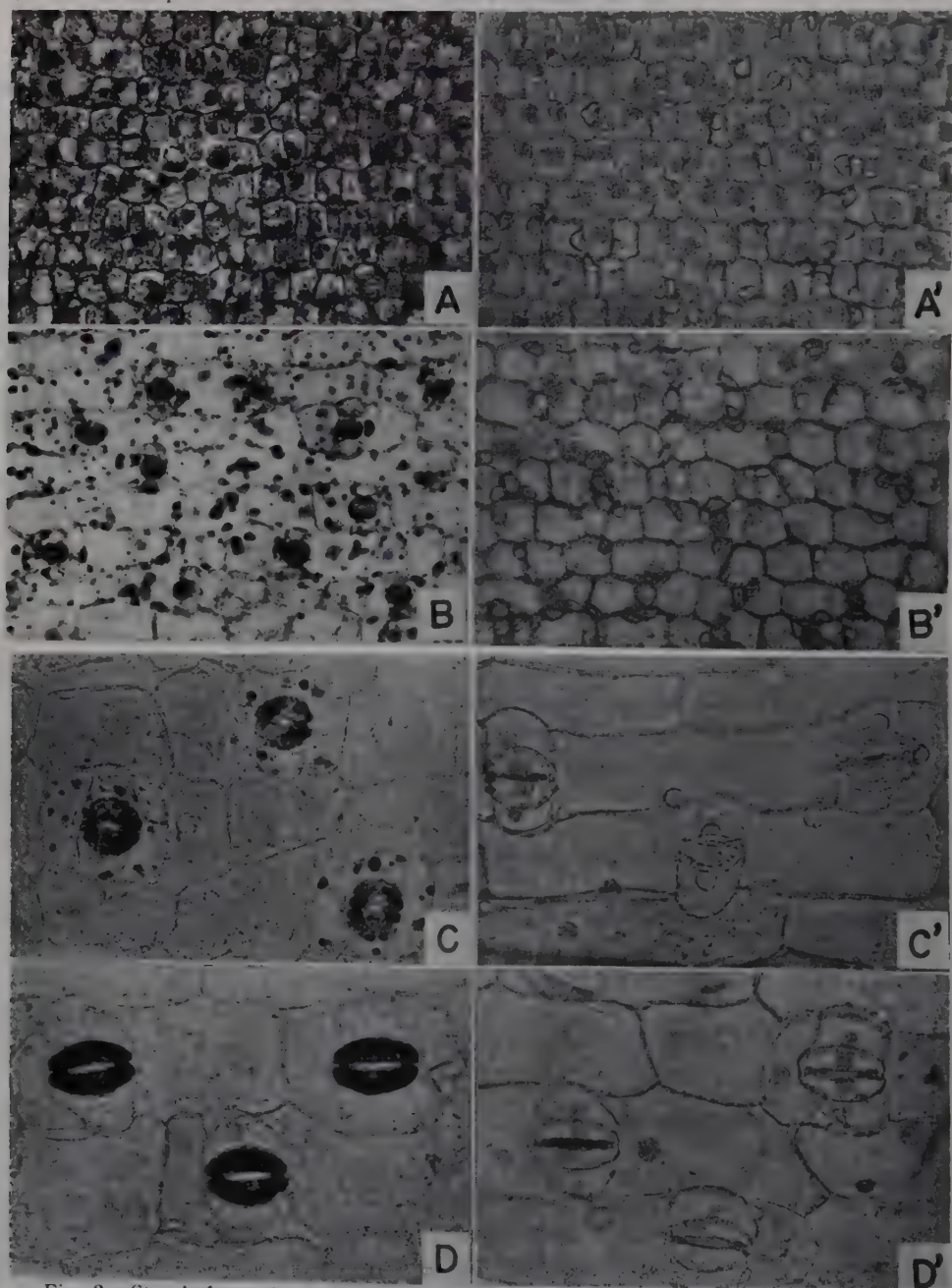


Fig. 2. Starch formation in parts a, b, c and d of leaf epidermis of *Tradescantia reflexa* treated with G-1-P solution. Stained with iodine (\times ca. 300). A~D, treated with G-1-P. A'~D', untreated with G-1-P.

of amylose type turned blue with iodine solution (Fig. 2A). This starch is the product of phosphorylase action in the tissue, because it was not detectable in the control (Fig. 2A').

In the stage of stomatal mother cells in part b of Fig. 1, phosphorylase action was detectable in every cell of the tissue, but a strong action was found in the mother cells and a fairly strong action was also seen in the subsidiary cells surrounding the mother cells (Fig. 2B). In the control tissue, however, there was no starch formation (Fig. 2B').

In part c, in which guard cells and stomata are formed by the division of stomatal mother cells, starch formation, i.e., phosphorylase action was remarkable in guard cells and still remained in subsidiary cells though weaker than that in the former stages (Fig. 2C). In the control tissues no starch formation was observed (Fig. 2C').

In the epidermis which was at perfect maturation (part d in Fig. 1) phosphorylase action was strong only in guard cells and not detectable in subsidiary and other epidermal cells. By the way, some guard cells at stage d contained starch grains a priori, so epidermal tissues containing no starch as shown in Fig. 2D' were selected for the present use.

The localization of phosphorylase was also examined in transections of young and grown parts of a leaf blade. In the young part (part a in Fig. 1) strong action of phosphorylase was found in every epidermal cell rather than in mesophyll cells (Fig. 3E), but in the older part (part d in Fig. 1) the enzyme action was found only in mesophyll and guard cells, and not in epidermal cells (Fig. 3F).

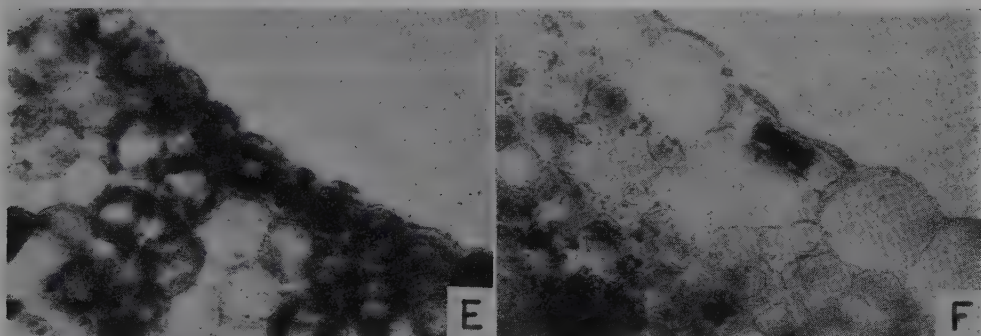


Fig. 3. Transections of a leaf blade of *Tradescantia reflexa* treated with G-1-P solution. Stained with ionine. (\times ca. 300). E, young epidermis. F, mature epidermis.

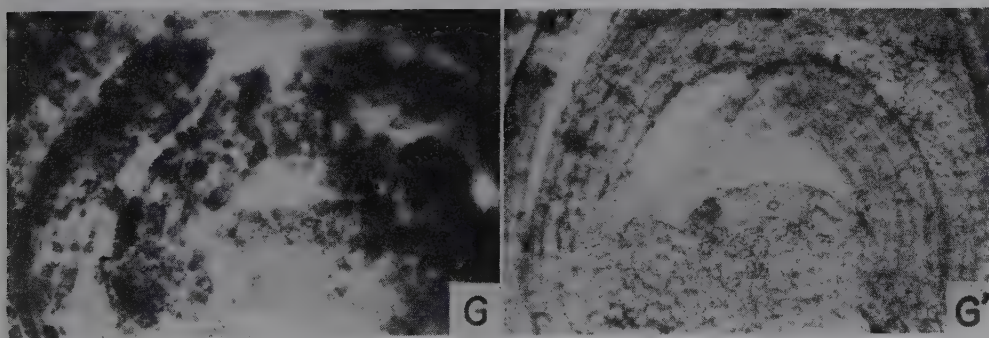


Fig. 4. Starch formation in shoot apex of *Tradescantia reflexa*. Stained with iodine. (\times ca. 300). G, longsection of vegetative shoot treated with G-1-P. G', control.

The strong action of the enzyme was also found in vegetative shoot apex of *Tradescantia*: in tunica, corpus and leaf primordia (Fig. 4 G).

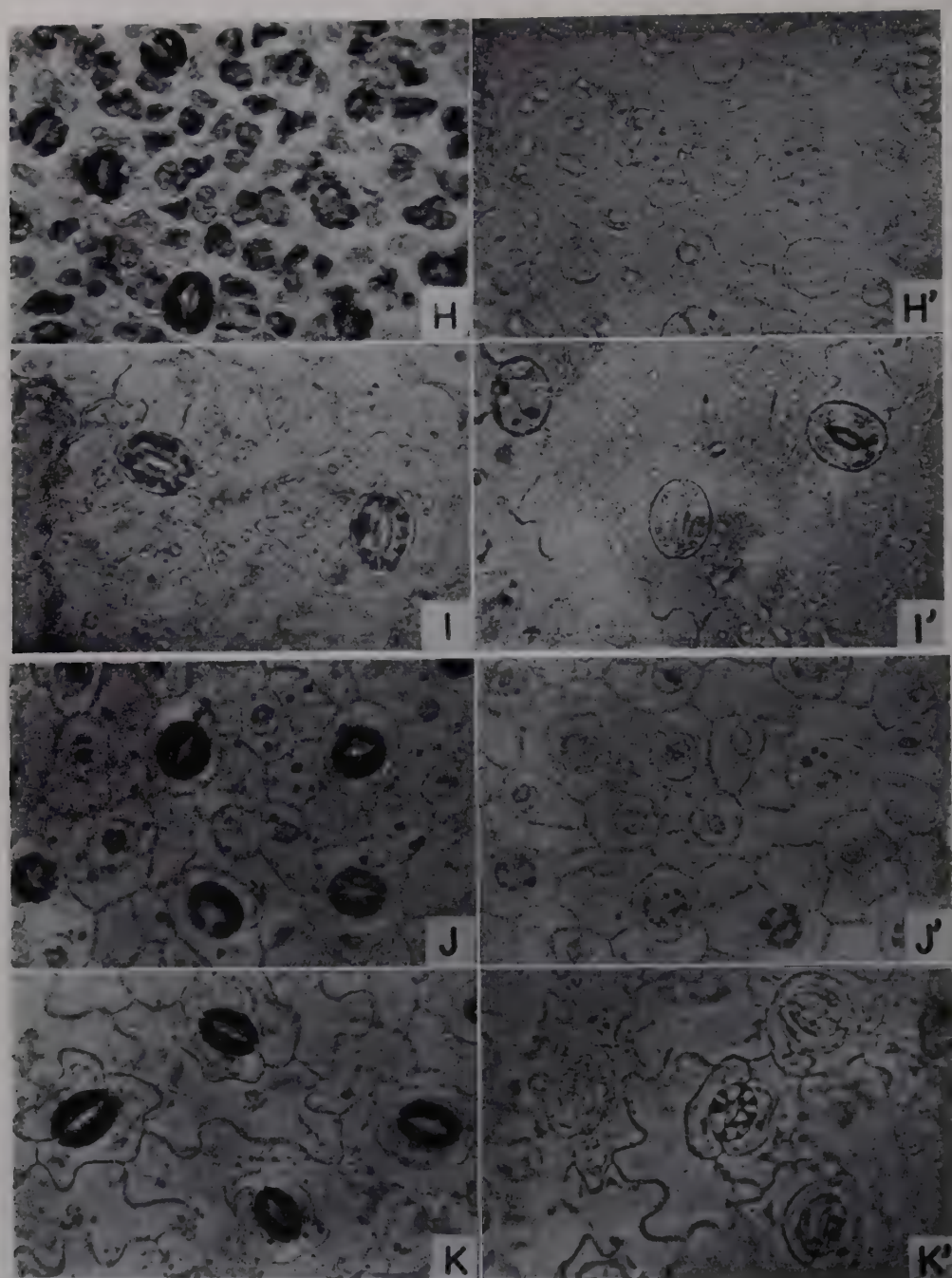


Fig. 5. Starch formation in leaf epidermis treated and untreated with G-1-P solution. Stained with iodine. (\times ca. 300). *Datura tatula*.....H, H', young epidermis; I, I', mature epidermis. *Vigna catianga*....J, J', young epidermis, K, K' mature epidermis.

Experiment in *Datura tatula* and *Vigna cati*ang Phosphorylase action in the leaf epidermis of *Datura tatula* and *Vigna cati*ang was also investigated (Fig. 5). In *Tradescantia* rather regularly zonal distribution of stomatal cells with respect to maturity was found along the blade axis, while in *Datura* and *Vigna*, as is the case for most dicotyledons, no such regularity was found and different developmental stages of stomata were mixed in a mosaic fashion, and fully mature guard cells often appeared adjacent to stomatal mother cells.

Starch formation was observed in all young epidermal cells (Fig. 5 H, J), but only in guard cells in mature epidermal cells (Fig. 5 I, K).

Discussion

The location of phosphorylase in epidermal cells other than guard cells has little been reported (Yin and Tung^{2,6}), Alvin⁷), Maruo and Nakamura⁸), Ono^{9,10}), Fukuda and Kato¹¹)).

In this experiment the phosphorylase action was examined in the leaf epidermis of various developmental stages of *Tradescantia reflexa*, *Datura tatula* and *Vigna cati*ang. In the early stage of development a strong action of the enzyme was found in every epidermal cell. With the maturation of the tissues the enzyme action became stronger in stomatal cells and weaker in the other epidermal cells until in fully mature epidermis it was found exclusively in guard cells.

According to Kato and Kato³), in the leaf epidermis of *Phytolacca americana* and *Datura tatula* sometimes a remarkable phosphorylase action was detectable not only in guard cells but also in other epidermal ones. In view of the present findings it seems probable that the leaf epidermis tested by these authors was not enough mature.

Yin and Sun¹) studied the distribution of phosphorylase in germinating seeds, e.g. those of soybean and found that the enzyme was present in root tip, young leaf and stem tip. Aimi and Kodera¹²) reported that the enzyme action was rather strong in young roots, and weak in older roots and leaves of germinating wheat plants.

In *Tradescantia*, as shown above, the strong action of the enzyme was found in various parts of vegetative shoot apex, i.e., tunica, corps, and protoderm and mesophyll of primordial leaves, whereas the epidermis of grown-up leaf contained it only in its guard cells.

These results are consistent with an idea that the phosphorylase action may take a part in the regulation of osmotic values of the plant cells, which would associate with stomatal movements in mature guard cells or with growth in young cells.

Summary

1) Phosphorylase action in the epidermal tissues isolated from growing leaves of *Tradescantia reflexa*, *Datura tatula* and *Vigna cati*ang was investigated. The tissues were incubated with G-1-P, and starch formed was detected with iodine reaction under a microscope.

2) In protodermal tissue a remarkable phosphorylase action was detectable in every cell.

3) With the maturation of epidermal tissue, the phosphorylase action in differentiating guard cells or stomatal mother cells became stronger, while in other epidermal cells it became weaker until it completely disappeared.

4) Meristematic shoot apex cells which contained no starch showed positive phosphorylase action.

5) It was presumed that phosphorylase action took an important role in the regulation of osmotic values of plant cells, which might be related to the growth of the young tissue as well as to the movement of stomata in leaf epidermis.

The author wishes to express his gratitude to Emer. Prof. Dr. Yasona Fukuda and to Prof. Dr. Tetsuo Fujita for their guidance and advice in this investigation.

References

- 1) Yin, H. C., and Sun, C. N., Science **105**: 650 (1947). 2) —, and Tung, Y. T., *ibid.* **108**: 87 (1948). 3) Kato, I., and Fukuda, Y., The 17th Annual Meeting of the Botanical Society of Japan (1952). 4) Konagamitsu, Y., and Ono, H., Sieboldia **2**: 131 (1959). 5) Stocking, C. R., Amer. Jour. Bot. **39**: 283 (1952). 6) Yin, H. C., Plant Physiol. **2**: 103 (1949). 7) Alvin, P., Amer. Jour. Bot. **36**: 781 (1949). 8) Maruo, B., and Nakamura, M., Jour. Agr. Chem. Soc. Japan **25**: 5 (1951). 9) Ono, H., Bot. Mag. Tokyo **66**: 103 (1953). 10) —, *ibid.* **66**: 182 (1953). 11) Fukuda, Y., Kato, I., Jour. of Science of Hiroshima Univ., Series B, Div. 2 (Bot.) **7**: 11 (1955). 12) Aimi, R., and Kodera, T., The 18th Annual Meeting of the Botanical Society of Japan (1953).

摘 要

加藤勇夫：植物における phosphorylase 作用の研究 I. 気孔発生過程の表皮組織における phosphorylase 作用

1) 多くの植物で気孔の孔辺細胞には顕著な phosphorylase 作用が検出されるが、孔辺細胞と同じく原表皮組織から発達した一般表皮細胞では通常この酵素作用を認めない。本実験では、发育過程における葉の表皮組織で、これらの兩種細胞における phosphorylase 作用の消長をムラサキツユクサ、ヨウシュチョウセンアサガオおよび、ササゲを材料として顕微化学的に検討した。

2) 原表皮組織には自然状態ではでんぶんの存在を認めないが、G-1-P 溶液に浸しておくと、組織のすべての細胞が顕著なよう素反応（でんぶん形成）を示す。

3) 組織の生長につれて、そこに分化した孔辺細胞では phosphorylase の作用はしだいに強まるが、その他の表皮細胞では逆に弱まってゆき、生長の完成した表皮では孔辺細胞にのみ phosphorylase の作用が認められる。

4) 茎の生長点の付近にある若い組織には、自然状態ではでんぶんの存在を認めないが、組織の G-1-P 液処理によって茎の先端部および葉原基の組織全体に顕著な phosphorylase 作用が検出される。

5) 気孔の孔辺細胞における phosphorylase 作用はこの細胞の滲透圧変化に関与することによって気孔開閉に寄与するといわれるが、幼細胞の含有する phosphorylase は、同じく滲透圧変化を介して生長に寄与するのではないかと考えられる。(広島大学教養部生物学教室)

ゴマにおける葉の背腹性に関する実験

埴

順*

Jun HANAWA*: Experimental Studies on Leaf
Dorsiventrality in *Sesamum indicum* L.

1961 年 2 月 21 日受付

葉原基ははじめ円丘状の隆起として発生する。その後の發育において背腹構造をとり平面状の展開をとげる。しかしある条件のもとでは、原基は背腹相称の葉のかわりに放射相称の器官として發育することがある。実験的につくりだされた放射相称器官は、これまでにいくつかの場合に報告されている。Snow と Snow (1939¹⁾) は *Epilobium hirsutum* の生長点を対角線の方に二分したのち、その小さい方から radial leaf をえた。また彼ら²⁾はごくわかい、生じたばかりの葉原基が茎頂からきりはなされると、しばしば放射相称に發育することを見いだした。Wardlaw (1945³), 1947⁴), 1949a⁵) は *Onoclea sensibilis* で茎頂がよわまったとき、あるいは *Dryopteris* でわかい生じたばかりの葉原基を茎頂から分離すると、錐状の器官が生ずることを見た。Wardlaw (1949b⁶)) はまた *Dryopteris aristata* で、葉の発生予定位置が茎頂から分離されると、葉のかわりに芽が生ずることをみだし、さらに Cutter (1954⁷)), Wardlaw (1955⁸)) はおなじ植物で、葉原基を茎頂から分離、または部分的に分離することによって、それを芽として發育させることに成功した。Sussex (1951⁹), 1954¹⁰), 1955¹¹)) は *Solanum tuberosum* の茎頂から葉形成予定位置 (I_1) を分離して放射相称器官 (centric organ) をえた。Soma (1958¹²)) は *Euphorbia lathyris* の茎頂を二分したのちに centric organ の発生を観察した。著者はゴマの幼芽を二分したのちに、その小さい方

の半分から杯葉が生ずることを観察した¹³)

Sussex^{10, 11}) は彼の実験結果ならびに他研究者のえた centric organ についての考察から、葉の背腹性は頂端分裂組織からくる効果によって決定されると結論した。Wardlaw⁸) も頂端分裂組織による支配を考えた。しかし Snow と Snow (1954a¹⁴)) は Sussex の結論には疑問があるとのべた。彼ら自身の、おなじ *Solanum* での実験は、Sussex のとかなりの不一致を示した。そこで彼らは centric organ はよわめられた葉形成部域、または必要最小限の大きさを満足しない部域から生じ、葉の背腹性は茎頂によってひきおこされるのではないと考えた。彼らによれば、必要な面積が茎頂の上にえられるならば、葉原基はそれ自身の内在的要因によって背腹性を発現することになる。これらのたがいに矛盾する考え方については議論された (Snow, 1954a, b^{14, 15}); Sussex, 1954, 1955^{10, 11})) こともあるが、背腹性の原因についてはまだ決定的な説明は与えられていない。この問題については今後の研究が必要である。ここに、ゴマの第 1 葉における、この問題についての実験結果を報告する。

材料と方法

ゴマ *Sesamum indicum* L. の第 1 葉 (普通葉) の原基は胚形成の途上、授粉後 11 日ごろに発生をはじめ、胚が熟すまでにその後 10 日以上を経過するが、熟した胚にあっても、ごくわかい發育段階 (buttress stage) にとどまっている¹⁶)。種まき 1 日後には原基はわずかに隆起をますだけであるが、その後は急速に生長し、3 日後には高さ 200~250 μ と

* Department of Biology, Faculty of Science, Tokyo Metropolitan University, Tokyo. 東京都立大学理学部生物学教室

なり、背腹構造があきらかになる。前報¹⁷⁾にのべたように、種まき1日後に、茎の中心を通る面で（すなわち放射方向に）、第1葉原基を二分すると、原基の両半分からそれぞれ正常な葉が発育した。その場合、背腹性の原因として三つのことがあげられる；(1)、この発育段階の葉原基は、内部的に未分化で、その半分の量があれば完全な葉をつくる能力がある。そのため自律的に背腹葉になった；(2)、原基は、放射方向に二分されたから茎頂との連絡が保たれて、そのため茎頂からの効果をうけて背腹葉になりえた；(3)、(1)とは逆に、この段階ですでに背腹性が確立されていた。そして放射方向での二分割はその体制を乱すことがなかった。

これら三つの仮定は、原基を種々の発育段階で、接線方向に二分割するか、あるいは茎頂から分離することによって、たしかめることができよう。そこでつぎのように、茎頂と葉原基に対して切りこみがなされた。すなわち、切りこみⅠ：茎頂の中央；切りこみⅡ：葉原基の向軸側のへり；切りこみⅢ：葉

原基の中央（第1図および第1表）。

手術は75倍の双眼顕微鏡下で、ネジ式微動操作器をつかってなされた。種子を水に15~30分つけたのち、種皮をとりぞいて胚を取りだした。裸にした胚を、ペトリ皿の中にしいた、しめった濾紙の上に置き、約30°, 6000 luxの蛍光灯による継続照明のもとで“発芽”させた。種皮をとりぞいた直後、ならびに24時間および48時間培養した胚の茎頂または葉原基を、第1図および第1表に示したように手術した。生長点を露出させるために子葉の1枚をとりぞいたが、この処置によって幼植物の発育はほとんどさまたげられなかった。手術された胚は前とおなじ条件下でそだてられた。観察は手術後3日目に双眼顕微鏡下でなされた。組織標本作製のために、観察ののち、いくつかをとってFAAで固定し、8~10μに切り、デラフィールドのヘマトキシリンでそめた。

観 察

1. 切りこみⅠ

切りこみが茎頂の中央になされると、第1葉はすべて正常に発育した。1日間培養した胚の手術ではすべての第1葉は完全な背腹性をしめした（第3図G）。これに対し休眠胚の手術では、背腹性の葉のほかに、少数の棍棒状の形成物、いわゆる *centric organ* が生じ、また円柱状の葉柄の先端に小さな葉片をつけた葉も生じた（第2表）。これらの異常形態はおそらく切りこみが茎頂の正中面をそれたために生じたと考えられる。それについては後で論ずる。なおこれらの異常形態は次ののべる切りこみⅡによって得られたものとおなじであるから、そこでくわしく記す。

2. 切りこみⅡ

休眠胚の手術でも1日後の胚の手術でも、この切りこみによって葉原基はすべて多かれ少なかれ背腹性を減少し、あるいは完全に失った。2日後の手術では背腹性をたもつ率が、他の二つの時期で手術したばあいよりも高かった。少ない割合のもののはかなりよく葉片を展開した（第2図A, 第3図C）。しかしそれらにおいても正常のものとくらべると、葉片部がやや小さく、葉柄部がより長く、全体の高さは正常のものよりやや高くなった。この葉柄部の断面をみると、正常なものにくらべて丸みをおび、背

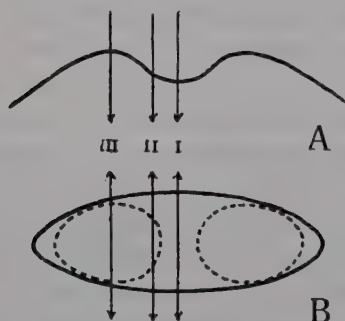


Fig. 1. Diagrams showing the shoot apex of *Sesamum* embryo and the positions of incision. Incision I, in the median plane of the apex. Incision II, along the adaxial border of the leaf primordium. Incision III, in the middle of the leaf primordium. A, side view. B, apical view.

第1表 切りこみの時期と位置

時 期	位 置
休 眠 胚	Ⅰ, および Ⅱ
種まき後 24 時間	Ⅰ, Ⅱ, および Ⅲ
種まき後 48 時間	Ⅱ

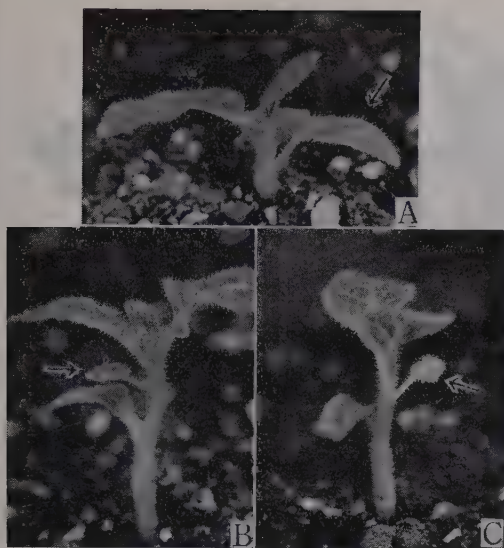


Fig. 2. Three types of more or less laminated leaves (indicated by arrows) which developed after the incision II on the 1 day-cultured embryos. A, nearly normal dorsiventral leaf. B, a leaf with the funnel-shaped lamina and the nearly centric petiole. C, a leaf with the spatulate lamina. All grown for 3 weeks after the operation. ca. $\times 1$.

腹性の減少をしめすものが多かった。これらの葉は、種々の程度に背腹構造の減少をしめしたが、かなり大きな葉片を展開したから、背腹葉とよぶのが適当であろう。

背腹性をもっと強く減少した場合には、先端にわずかの葉片をつけ、その下方大部分は円柱状となった(第2図C, 第3図B, E)。先端の葉片部は時には杯状になることもあった(第2図B)。これらの葉の葉柄部の断面をみると、輪廓はほとんど円形となっているが、組織的には向軸側と背軸側との間に、皮層と表皮の細胞の大きさと空胞化の程度とにおいて差を認めることができる。その維管束系は並立維管束とはならず、包囲維管束的のもの(管状または放射中心柱に近い維管束系)となった(第3図E)。

これら小葉片をもった葉は、さきにもべた背腹葉と、つぎにもべる完全な centric organ との中間的段階のものである。

葉原基分離の効果のもっともいちじるしい場合は葉原基は完全な放射相称となり、棍棒状あるいは錐

状となった(第3図A)。この器官は太さ約 0.2 mm., 長さ 0.5~1 mm. で、葉片はまったく展開しない。横断面でみられる構造は、まったく背腹性がなく組織は一様に空胞化した細胞よりなる。維管束系はきわめて簡単で、2, 3 個のやや厚膜の小型の細胞が中央に認められるだけである(第3図D, F)。

なお分離された原基の伸長生長は、手術後 3 日目に観察したときには、一般に intact の原基より進んでいた。しかしその後の生長は限られ、最後には正常のとくらべて微小な形成物におわった。

このように、分離された葉原基はすべて多かれ少なかれ背腹性がよまった。ほとんど正常なものから完全な centric organ にいたるまで、背腹性の漸次的減少に応じてそれらを系列にならべることができる。しかし記録のために、それらを一応つぎのように区分して、その比率を第2表にあげる。(1)背腹葉：観察のとき、葉片部の大きさが正常のもの半分以上あるもの；(2)中間型：葉片の大きさが正常のもの半分以上より小さいもの；(3)centric organ：葉片をまったく欠き、棍棒状または錐状のもの。

3. 切りこみⅢ

この切りこみによって葉原基は向軸側と背軸側とに二分された。

背軸側の半分からは完全な centric organ のみを形成した(第4図)。退化してなにも生じない率も高かった(第2表)。これらの centric organ は一般にまえにのべた切りこみⅡによって生じたものよりもさらに微少で、より完全に centric であった。維管束として、中央にごくわずかに分化した小さい細胞の集まりをもつものもあるが(第4図E)、多くの場合、器官全体が空胞化した細胞のみからなり、中軸的な組織はなにもなかった(第4図D)。前者の場合は切りこみが原基の中央よりもいくぶん向軸側に寄ったために、すなわち切りこみⅡに近い位置に切りこみがなされたために、生じた器官は比較的大きく、かつその中に維管束分化の傾向を示したのである。

向軸側の半分は、正常のものと同程度に生長はよめられたけれども、背腹構造は保たれた(第4図A-E)。

以上にのべた三つの時期と位置における切りこみの結果は第2表に総括してある。

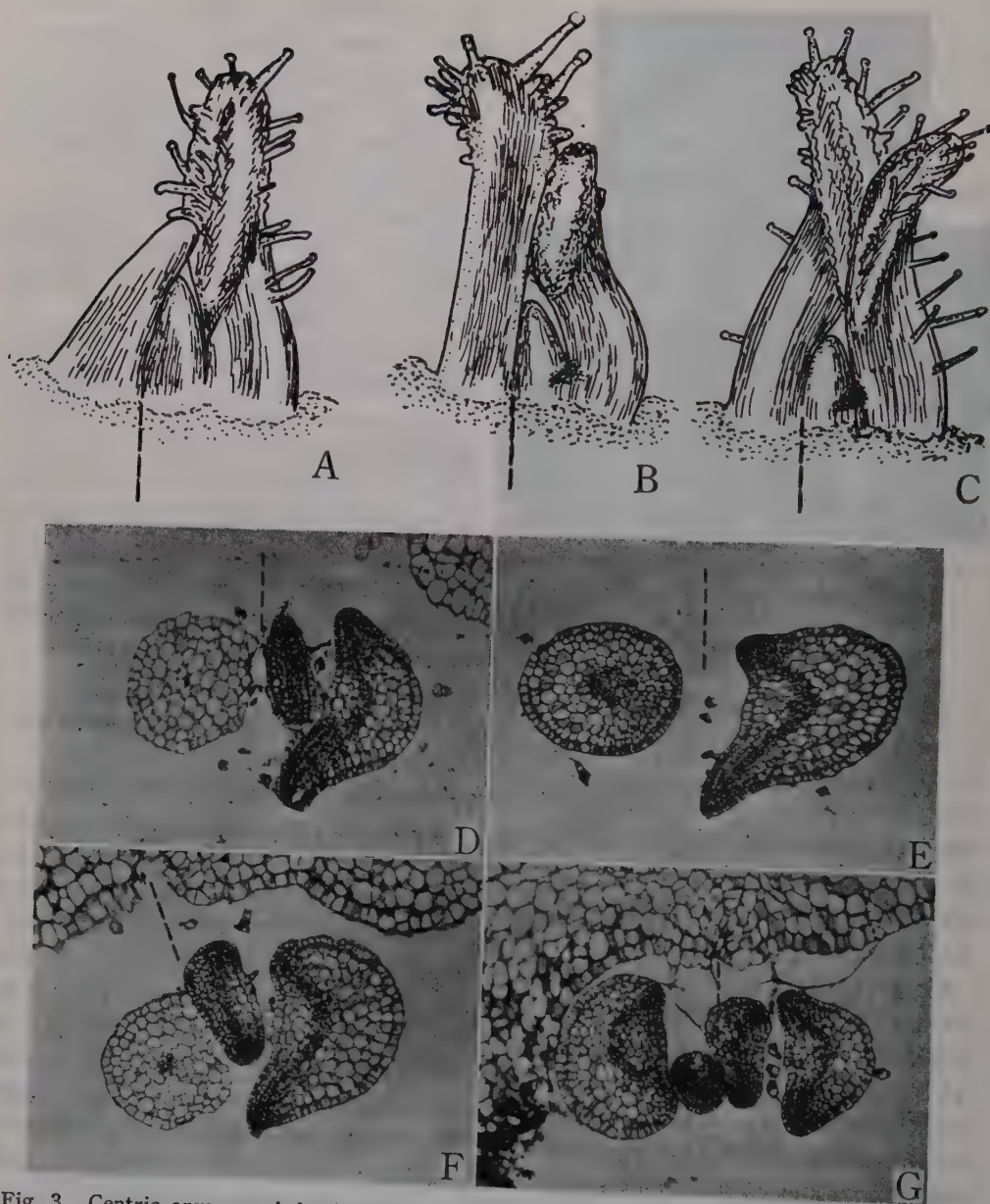


Fig. 3. Centric organs and dorsiventral leaves that developed after the incision on the 1 day-cultured embryos. A-F; incision II. G; incision I. Broken lines indicate the plane of incision. A; awl-shaped centric organ. C; nearly normal dorsiventral leaf. B; intermediate type between the centric organ and the dorsiventral leaf. D and F; transverse sections of the centric organs, together with the intact normal leaf of the original pair and the next leaf appearing oppositely to the first. E; transverse section of the petiolar region of the intermediate type, the structure of which is almost centric. G; transverse section of the two dorsiventral leaves, both accompanied by the regenerated shoot respectively. A-C; $\times 37$, D-G; $\times 90$ (A-C; traced on photographs).



Fig. 4. Centric organs that developed after the incision III on the 1 day-cultured embryos. Broken lines indicate the plane of incision. A; slight swelling, formed from the abaxial half of the bisected primordium. B and C; centric organs developed from the abaxial half of the bisected primordium. All the adaxial halves developed to the dorsiventral leaves. D; transverse section of one of the smallest centric organs. E; transverse section of larger centric organ. A-C; $\times 37$, D-E; $\times 90$ (A-C; traced on photographs).

Table 2. Effects of incision at different stages and in various positions upon dorsiventrality of the first leaf primordium.

Operation stage	Resting embryo		24 hours after sowing				48 hours after sowing
	I	II	I	II	III*		II
Position of incision					ab.	ad.	
Dorsiventral (%)	80.0	13.6	100	20.6	0	100	52.6
Intermediate (%)	13.0	18.2	0	25.0	0	0	5.3
Centric (%)	7.0	54.6	0	39.7	27.7	0	26.3
Degenerated (%)	0	13.6	0	14.7	72.3	0	15.7
Total number	15	22	15	68	18	18	19

* ad: adaxial half, ab: abaxial half.

考 察

切りこみ I, II, III の結果を、それぞれ三つの時期について比較することにより、つぎのような推論が可能であろう。

1. 切りこみ I について。

休眠胚の生長点の手術では、わずかながら *centric organ* およびそれに近い葉が生じたが、1日培養した胚の手術ではそのような器官は形成されず、葉原基はすべて背腹葉となった。切りこみ I は実質的には葉の背腹性の発現をさまたげない。葉原基が頂端分裂組織の半分をわかちもっていることが、完全な背腹性の原因であろう。ただし、二つの時期での手術の結果の間には、今のべたような差がある。このことの原因としては、つぎのように原基の発育段階の差が考えられる。すなわち、1日培養した胚では休眠胚よりも葉原基の背腹性の分化が多少とも進んでいるのではなからうか。そのため、切りこみがすこし正中面をはずれて葉原基に付着した頂端分裂組織の分量が減少しても、葉原基は背腹葉になりえたのであろう。休眠胚においても、切りこみがすべて正しく茎頂の正中面にはいるならば、すべての葉は背腹性となるであろう。

したがって、上のような推論には、葉の背腹性の要因として、完全な1個の茎頂が必要なのではなく、ある程度の量の頂端分裂組織があればよい、という考え方がふくまれている。Wardlaw (1955⁹)) も、シダ植物において、茎頂の中心ではなく側部が葉の背腹性を決定することを示唆した。

2. 切りこみ II について。

この切りこみは休眠胚、1日および2日培養された胚の三つのものについてなされた。葉原基を茎頂から分離することの、背腹性発現に対する阻害的影響はあきらかである。このばあいも、切りこみ I についてのべたのと同じように、葉原基の発育段階の差異と、実際の切りこみの、計画位置からのずれとが、形成される器官を多様化している。切りこみが茎の中心の方向へずれて、原基が頂端分裂組織の一部を分けあたえられるならば、背腹的傾向はつよまるであろう。それは切りこみ I において実際の切りこみが中央より側方へずれた場合と同じ結果となる。これらの場合は、頂端分裂組織の量の大小によって背腹性の強弱が支配されると想像される。

切りこみが逆に外方にずれたときは強度の *cen-*

tric organ が生ずるであろう。それはつぎにのべる切りこみ III と同じ効果となる。

葉原基は発育段階に応じて背腹性の決定も強まっていると考えられる。したがって、同じ程度の切りこみ位置のずれが各時期での手術の際におこるとすれば、後の時期ほど背腹葉の形成率は増加し *centric organ* の形成はへるであろう。第2表に示すように、実験の結果はそのことを証明する。しかし、48時間までの間には、原基にある程度の背腹性が生じているとしてもまだ確定していないため、ある割合の原基は、切り込みの位置によっては、それを失って *centric organ* となったのであろう。Cutter (1954⁷)) は *Dryopteris aristata* において、葉原基をプラストクローン初期に分離するとそれが芽になる率が高く、おそいプラストクローンの時期では葉として発育する率が高くなることをみた。これは葉原基内の分化の差によるものであることが示された。また Sussex (1955¹¹)) は *Solanum tuberosum* において、葉原基 P_2 や P_3 がその葉腋にそって頂端分裂組織から分離されると背腹葉になるが、この手術が生じたばかりの原基 P_1 に対してなされると、分離された原基のうち少数のものは退化し、いくつかは背腹葉となり、他は *centric organ* として発育することを観察した。Sussex はさらに I_1 を、その発現のとき以後種々の時期に、茎頂から分離する手術をおこない、それを *centric organ* に発生させたが、24~36 時間以後は、葉原基は分離されても背腹性を失わないことをみだした。彼の実験条件のもとでは *Solanum* の1プラストクローンは約1日であると彼はのべているから、背腹性の固定までに、原基の発生後1プラストクローンを経過したとみてよいであろう。ゴマでは1プラストクローンは約4日であるから、種まき後48時間までの期間はプラストクローンの初~中期にあたる。この時期には、切りこみ II によって分離された第1葉原基は、Sussex によって分離された *Solanum* の P_1 と同じように背腹葉となったり、*centric organ* になったりした。しかし、手術の時期がおそくなるにつれて背腹葉の形成率は高くなった。そしておそらく、Sussex がしめしたように、種まき4日後、すなわち1プラストクローン経過後には、分離された原基はすべて背腹葉となるであろう。本実験によって、*Solanum* よりもはるかにおそい速度で進行す

る、ゴマの葉の背腹性分化の経過がみいだされたといえる。

3. 切りこみⅢについて。

さきにもべたように、培養1日の胚の第1葉原基を放射方向に二分すると、各半分から完全な葉が生じた。そして、この時期の原基は葉形成の能力において同等な細胞からできていると考えられた¹⁷⁾。したがって葉の背腹性が原基自身の内因によってひきおこされるものならば、二分割の方向にかかわらず同じ結果を期待することができよう。しかしすでに上にのべてきたことから、そのような期待が不可能であることはあきらかである。さらに、切りこみⅢの結果が示すように、接線方向に二分された原基の、背軸側の半分はすべて完全に centric となった。これに反し向軸側の半分はすべて背腹葉となった。この結果は葉の背腹性に対する頂端分裂組織の支配をもっとも端的に示すものであろう。

しかし同時に、切りこみⅠおよびⅡの実験によ

て示されたように、この時期の葉原基にはわずかながら背腹性の分化がおこっているらしい。とすれば、背軸側の半分が背腹性を失ったのは、茎頂の支配がたちきられたことのほかに、原基自身の発生能の低下にもよると考えられる。

結 論

以上の実験結果から、本実験の範囲内では、葉の背腹性を決定する主要な要因は、頂端分裂組織からくるなんらかの効果であって、葉原基自身の内在的要因は、たとい存在するとしても、前者にくらべてきわめてよいいか、あるいはその機能において二次的なものであると推論される。また、発育にともなう葉原基の中に背腹性の分化が進行するが、完全な決定にいたるまでの期間では、その背腹性分化は可逆的であって、実験条件によって減少または消失させられる。完全な背腹性の決定にはおそらく1プラストクロンの発育を要するであろう。

文 献

- 1) Snow, M., and Snow, R., Phyl. Trans. Roy. Soc. London B 225 : 63 (1935).
- 2) —, and —, New Phytol. 41 : 13 (1942).
- 3) Wardlaw, C.W., Ann. Bot. 9 : 383 (1945).
- 4) —, Phyl. Trans. Roy. Soc. London B 232 : 343 (1947).
- 5) —, ibid. 233 : 415 (1949a).
- 6) —, Growth (suppl.) 9 : 93 (1949b).
- 7) Cutter, E.G., Nature 173 : 440 (1954).
- 8) Wardlaw, C.W., ibid. 175 : 115 (1955).
- 9) Sussex, I.M., ibid. 167 : 651 (1951).
- 10) —, ibid. 174 : 351 (1954).
- 11) —, Phytomorphol. 5 : 286 (1955).
- 12) Soma, K., Jour. Fac. Sci. Univ. Tokyo Sect. III, Bot. VII : 199 (1958).
- 13) Hanawa, J., and Ishizaki, M., Sci. Rep. Fac. Lib. Arts and Educ. Gifu Univ. 1 : 55 (1953).
- 14) Snow, R., and Snow, M., Nature 173 : 644 (1954a).
- 15) —, and —, ibid. 174 : 352 (1954b).
- 16) Hanawa, J., Bot. Mag. Tokyo 73 : 369 (1960).
- 17) —, ibid. 72 : 425 (1959).

Summary

Effects of isolation and bisection of the leaf primordium by tangential incisions upon its dorsiventral development were studied for the first leaf of *Sesamum indicum* L. Incisions were made on the shoot apices of three stages, i. e., dormant, 1 day- and 2 day-cultured embryos, and in three positions, i. e., in the median plane of the shoot apex, along the adaxial border of the leaf primordium and in the middle of the leaf primordium.

When the incisions were made in the median plane of the shoot apices of the dormant and the 1 day-grown embryos, the leaf primordia developed dorsiventrally. When the leaf primordia were isolated along their adaxial border from the apical meristem, they developed as centric organs in higher proportions after the operation at earlier stages than after that at later stages. When the leaf primordia were bisected tangentially, the abaxial half developed only as a centric organ, whereas the adaxial half formed every time a dorsiventral leaf.

From above results, it is inferred that the principal factor determining leaf dorsiventrality is the effects from the apical meristem, and that the intrinsic factor, if any, of the leaf primordium itself may be very faint or of secondary nature in its function. Moreover, it is suggested that dorsiventrality may become gradually intensified in the course of plastochron and perfect establishment of dorsiventrality may be attained at the end of the first plastochron.

Miscellaneous Note

Kiyonobu TOYODA*: A Complementary Note on the Chlorophylls in Some Spermatophytic Seeds

豊田清修*: 種子中のクロロフィルについての補遺

Received August 10, 1960

In a previous paper¹⁾, the writer reported on the chlorophylls contained in some angiospermous seeds. Chlorophylls were further found in some spermatophytic seeds, which this paper refers to. The seeds investigated are as follows:

1. *Citrus erythrosa* Hort. ex Tanaka and *C. Kinokuni* Hort. ex Tanaka.
2. *Euonymus japonicus* Thunb.

In the fruit of *E. japonicus*, there exist 1-5 seeds which have red arils. The seed has white seed-coat and white albumen, and a green embryo located in the inner part of the seed was used.

3. *Rhaphiolepis umbellata* Makino and *R. umbellata* var. *Martensii* Makino.

The outer layer of the pericarp of *R. umbellata* is dark-purple and the inner layer is green. The seed-coat is brown and covers the light-greenish cotyledons. The pigments in the cotyledons were studied.

4. *Ginkgo biloba* L.

The seed of *Ginkgo* has a soft episperm and a hard endopleura, and the inner part is filled with yellowish-white albumen. A yellowish-green embryo located in the interior of albumen was used.

At first the green pigments were analyzed chromatographically in the same manner described in a previous paper¹⁾. Besides, lest the protochlorophyll should change into chlorophyll *a*²⁾, the experiments were carried out using the seeds after they had been soaked in boiling water for 5-10 minutes. In some cases, after the green parts of the seeds had been exposed to the sunlight for 1-2 hours, the pigments were studied to see the influence of light.

Table 1. The results of paper chromatography on the pigments in some seeds (at 16-25°).

		Rf value and color ^a			
1)	a) <i>Citrus erythrosa</i>	0.38 (YG)	0.80 (Y)		
	b) <i>C. Kinokuni</i>	0.35 (YG)	0.58 (Y)		
	Do. developed after exposing to the sunlight	0.27 (YG)	0.34 (BG)	0.52 (Y)	
2)	<i>Euonymus japonicus</i>	0.18 (YG)	0.21 (BG)	0.69 (Y)	
	Do. developed after soaking in boiling water	0.21 (YG)	0.80 (Y)		
3)	<i>Rhaphiolepis umbellata</i>	0.23 (YG)	0.29 (BG)	0.57 (Y)	0.97 (Y)
	Do. developed after soaking in boiling water	0.23 (G)	0.55 (Y)	0.73 (Y)	0.96 (Y)
	Do. developed after exposing to the sunlight	0.28 (YG)	0.35 (BG)	0.63 (Y)	
4)	<i>Ginkgo biloba</i>	0.52 (G)	0.80 (Y)		
	Do. developed after exposing to the sunlight	0.37 (YG)	0.44 (BG)	0.68 (Y)	0.95 (Y)

^a YG yellowish green; BG bluish green; Y. yellow.

* Fujisawa Higher School attached to Nihon University, Fujisawa, Kanagawa, Japan.

The paper chromatography was carried out using carbon tetrachloride and the results are shown in Table 1.

The chromatogram of the pigments of *R. umbellata* var. *Martensii* was similar to that of *R. umbellata*.

The absorption curves of the green pigments of those seeds dissolved in acetone were taken with the aid of a Beckman spectrophotometer, and the results are shown in Figs. 1 and 2.

From the results of these experiments, following conclusion may be drawn. The embryo of *Citrus erythrosa* has chlorophylls *a* and *b* judging from its absorption curves,

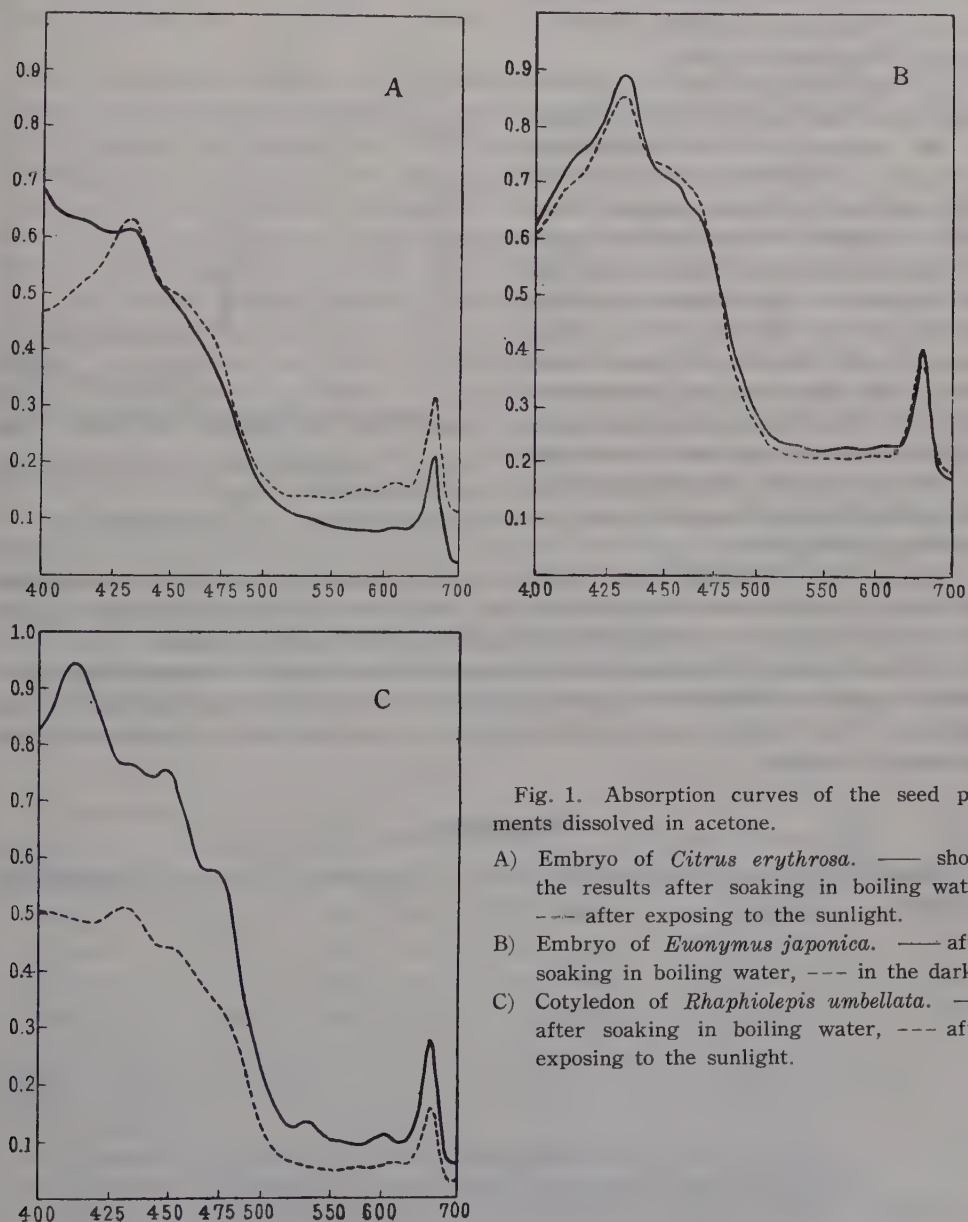


Fig. 1. Absorption curves of the seed pigments dissolved in acetone.

- A) Embryo of *Citrus erythrosa*. — shows the results after soaking in boiling water, --- after exposing to the sunlight.
- B) Embryo of *Euonymus japonica*. — after soaking in boiling water, --- in the dark.
- C) Cotyledon of *Raphiolepis umbellata*. — after soaking in boiling water, --- after exposing to the sunlight.

though it shows only the YG spot on the chromatogram. The embryo of *C. Kinokuni* also has chlorophyll *a* and a small amount of chlorophyll *b*.

The chromatography of the embryo of *Euonymus* was taken in two different ways; in the dark and after soaking in boiling water. A light BG spot which was found on the chromatogram of the untreated seed did not appear after soaking in boiling water. Little difference was, however, seen between the absorption curves of those samples. The embryo of *Euonymus japonicus* seems to contain chlorophylls *a* and *b*.

A light YG spot with a large Rf value was seen on the chromatogram of the pigment of the *Rhaphiolepis* cotyledons which had been extracted after soaking in boiling water. However, a similar YG spot did not appear for the seed exposed to the sunlight, and the exposure to light seemed to enlarge the area of the BG spot. It is, therefore, inferred that the YG spot having a large Rf value may be protochlorophyll. The illumination may have transformed protochlorophyll into chlorophyll *a*. The cotyledon of *Rhaphiolepis* seems to contain chlorophylls *a* and *b*, and protochlorophyll (Fig. 1-C).

The embryo of *Ginkgo* seems to have a large amount of carotenoids, protochlorophyll and a small amount of chlorophyll *a*. The results of chromatography indicate that protochlorophyll is changed into chlorophyll *a* by exposure to light.

The seed-coat of *Euonymus* is semitransparent and it may transmit a faint light. While, the seeds of *Rhaphiolepis* and *Ginkgo* seem to be non-transparent due to thick seed-coat. It is worth to notice that chlorophylls were identical for completely non-transparent samples.

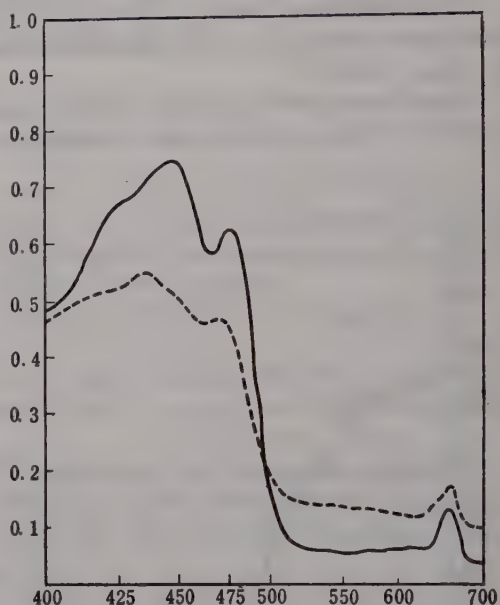


Fig. 2. Absorption curves of the seed pigments dissolved in acetone.

— *Ginkgo biloba*, --- *Citrus Kinokuni*.

References

- 1) Toyoda, K., Bot. Mag. Tokyo **72**: 159 (1959).
- 2) Hejnowicz, Z., Physiolog. Plantarum **11**: 878 (1958).

Short Communication

Tadashi HIRATA* and Kazuo FUJITA**: On Amino Acid Constitution of *Poncirus* Seed Protein***

平畑 規*, 藤田一夫**: カラタチの種子タンパク質の構成アミノ酸について

Received April 28, 1961

The properties of the proteins in the seeds were investigated with special reference to their amino acid composition. For detecting individual amino acids, high voltage paper electrophoresis has revealed to be most sensitive and reliable.

Mature seeds of *Poncirus trifoliata* Raf. were used as the materials.

Five g. of the material (eliminated the seed coat) were defatted with ethyl ether. The protein in the residual mass was extracted with 1/30 M. phosphate buffer (pH=7.0), containing NaCl in 10%. The protein in the extract was fractionated by salting out with ammonium sulphate. The flocculent mass of protein formed at each salt concentration was separated by centrifugation of the mixture. Each protein fraction thus obtained was dialyzed for 24 hours against the phosphate buffer, and then concentrated with carbowax. Each protein sample was hydrolyzed at 120° for 3 hours with ten volumes of 1/6 HCl and buffered with formic-acetic acid mixture (pH=1.5). Two μ l. of the sample was subjected to the electrophoresis (7 mA/cm., 100 V/cm.) for 15 min. in the formic-acetic acid medium (pH=1.5), cooled in a *n*-hexane bath. The spots of the constituent amino acids were detected with ninhydrine and determined by comparing with those of the pure samples of amino acids applied on the same sheet. The optical density was measured with a densitometer.

By comparing the density values of the amino acids in each hydrolyzate of the four protein fractions, the following results were obtained.

On the other hand, the mixture of the protein obtained in same way was subjected to the low voltage electrophoresis (0.5 mA/cm., 10 V/cm., sample 0.003 ml.) for 3 hours

Table 1.

Amino acid Sat. of (NH ₄) ₂ SO ₄	Lysine	Ar- ginine, His- tidine	Glycine	Alanine	Iso- leucine, Serine	Leucine	Proline	Phe- nylala- nine, Cystine	Aspar- tic acid
0 ~0.4	1.00	1.63	2.50	3.25	3.62	3.50	3.06	3.44	3.44
0.4~0.5	1.00	1.90	1.38	1.55	2.21	2.17	1.62	2.66	2.62
0.5~0.6	1.00	2.71	2.00	2.00	4.00	4.71	4.00	6.35	5.00
0.6~0.8	1.00	3.00	1.78	3.22	4.56	4.00	4.11	9.78	7.56
Distance of Cataphoresis (cm.)	19.7	18.8	16.0	14.4	12.0	11.7	11.0	10.0	9.2

* Department of Biology, Wakayama Medical College, Wakayama, Japan.

** Department of Chemistry, Wakayama Medical College, Wakayama, Japan.

*** Supported by the Grant in Aid for Scientific Research from the Ministry of Education.

in a medium of the phosphate buffer (pH=7.0). The spots of the protein were detected by B.P.B. dying, and the optical density was measured with a densitometer. The following Fig. 1 is graphical diagram of the distance of cataphoresis *vs.* the optical densities.

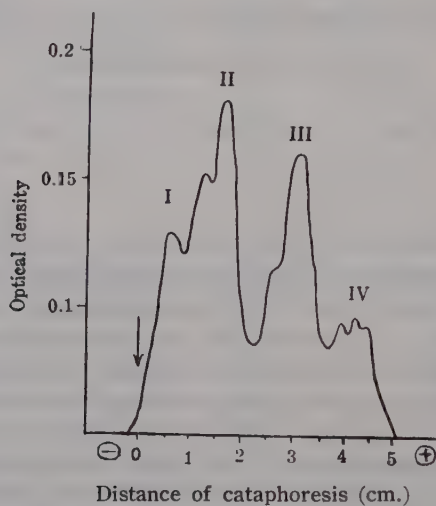


Fig. 1.

The relations between the four hydrolyzated protein fractions in Table 1 and the protein fractions (I, II, III, IV) in Fig. 1 are being investigated.

We express our hearty thanks to Prof. S. Kitamura and Mr. Y. Momotani for their advice.

抄 録

南極海域の植物プランクトンと
クロロフィル量

Burkholder, P. R., and Sieburth, J. M.: Phytoplankton and Chlorophyll in the Gerlache and Bransfield Straits of Antarctica. *Limnology and Oceanography* 6: 45~52 (1961).

南極大陸グレイアムランド（パーマー半島）の西にある Gerlache, Bransfield 両海峡の植物プランクトンとクロロフィル量の調査を、1958 年 12 月から 1959 年 1 月にかけて行なった。珪藻類 *Thalassiosira*, *Coscinodiscus*, *Eucampia*, *Synedra*, 黄色弁毛藻類 *Phaeocystis*, などに富む群落は Gerlache 海峡および Bransfield 海峡西域で観察された。渦弁毛藻類は、この海域では非常に少なかった。グレイアムランドの先端近くにあたる Bransfield 海峡の西域では、全深度にわたって、植物プランクトンはほとんど見いだされなかった。クロロフィル量の測定結果は、一般に、植物プランクトンの個体数の計数結果と一致した。クロロフィルの分布はいちじるしく不均一で、Bransfield 海峡北西域では $0.3 \sim 0.5 \text{ mg/m}^3$, Gerlache 海峡では $10 \sim 25 \text{ mg/m}^3$ で、最高値は約 27 mg/m^3 であった。この最高値は Long Island Sound で植物プランクトンが大発生する時期に得られた最高値(Conover, 1956)にはほぼ等しく、Gessner (1949) が示した理論的に可能な最高値に近いことは興味深い。また、北極海で得られた最高値 0.37 mg./m^3 (Apollonio, 1959) や北西太平洋親潮水域で得られた最高値 0.98 mg./m^3 (西条・市村) や California の La Jolla で得られた年間 $0 \sim 1.7 \text{ mg./m}^3$ (Graham, 1943) の範囲にはいるという報告よりも大きい。Ryther and Yentsch (1957) による生産の高い海域での値と一致する。クロロフィルの垂直分布を 5 地点で調査した結果、いずれも、表層に最高値があり、深度が増すにつれて、その減少がみられた。表層水中のクロロフィル量は、その値が 2.5 mg./m^3 以上の水域では、水深の浅いところのものでは多く、深いところのものほど少なかった。

(有賀祐勝)

二次遷移における物質生産

Odum, E. P.: Organic Production and Turnover in Old Field Succession. *Ecology* 41: 34~49 (1960).

放棄された作物畑の植物群落の二次遷移は、これまで群落の“構造面”ともいべきその種類組成の変化が主として研究されてきた。そして、優占種の交代や種数の増加は、当然群落の生産力の増大を伴うものと考えられ、Oosting (1956) は、群落が最大生産力をうるときに極相に達すると仮定した。Odum のこの研究は、アメリカ東南部の 27,000 ヘクタールにのぼるワタ、コムギ、トウモロコシ畑が 1951 年に放棄された後 7 年間にわたって行なわれ、遷移の“機能面”を強調して生産量測定をとり入れた。はじめの 3 年間に、一年生広葉である *Leptilon*, *Haplopappus*, *Heterotheca* などの優占種の活発な交代がおこり、優占種数も年ごとに増して群落は複雑になっていった。多年生の *Andropogon* は 5~7 年目に優占種となった。しかしながら、群落の生産量は、畑作時の肥料の影響が考えられた 1 年目と降雨の少なかった 3 年目を例外として、乾量で約 $300 \sim 350 \text{ g./m}^2 \cdot \text{year}$ の安定した値がつづいた。*Andropogon* の侵入は、群落の生産力を多少変化させたが、それは根の分布が土壌中のより深部へ達するために、一年生広葉が利用できなかった栄養塩類や水を獲得することによるものと考えられ、変化の幅はきわめて小さかった。同時に行なった予備的研究によって、マツの侵入も生産力を変えないことがわかったし、また土壌組成のちがいは、極端な場合をのぞけば、遷移の速度と種類組成にだけ影響し、群落の機能面への影響は少ないこともわかった。これらのことから、遷移は、一般に考えられていたように“生産力のちがいが生じる種の交代によって、段階的な小休止を経て進行する”のではなく、機能的には“生活形の各段階で安定しながら変化する”ものであると彼は述べている。ではなぜ群落内の優占種の頻繁な交代や、群落組成の複雑化がおこるのかという問題は、まだあきらかにされなかった。しかし、植物の生育期間のずれ、種間の競争、いや地などは、いずれもそれらの原因の一つに数えられる。

(牛島忠広)

大会関係のお知らせ

植物学会大会の前日、10月12日(木)午後1時から、東大前養賢堂会館で、酵母細胞討論会をひらきます(5月号で予告しました日時を、このように訂正します)。講演者とその演題は下記のとおりです。

倉石 衍(東北大・応微研): 酵母のビタミン欠乏培地中でのアンバランス・グロース

前田章夫(東大・教養): リボゾームの構造と機能

瀬野悍二(京大・植物): 酵母の耐銅性の遺伝

江夏敏郎(阪大・発酵工学): 酵母によるトリプトファン発酵

高橋俊明(醸造科研): 酵母の染色体地図

なお当日6時半からひきつづき懇親会をもちます。

酵 母 細胞 研 究 会

同12日(木)、午後3時から湯島聖堂内斯文会(お茶の水駅、聖橋口下車)で、菌学会を開きます。講演者とその演題は下記のとおりです。資料の供覧もあります。

コルフ博士: 世界における *Discomycetes* 研究の現状

小林義雄: 中国料理にあらわれる菌類

久内清孝: 中国食品談

中沢亮治: 中国の酒と酵母

なお会費は100円です。

菌 学 会

大会第一日の13日(金)、午後5時半から8時半まで、東大赤門横、本郷学上会館で分類学会をひらきます。なお会費は500円です。

分 類 学 会

同13日(金)午後6時半(夕食後)から8時ごろまで東大構内東大出版会集会室で植物細胞微細構造談話会(仮称)を開きます。

重 永 道 夫(奈良女子大): 分裂中の細胞の微細構造

川 上 襄(広島大・工): 菌類の電顕的構造

会費はいりません。くわしくは東大植物学教室の佐藤七郎にお問い合わせください。

同13日(金)午後5時から8時まで、東大構内山上会議所で、若い研究者が、おたがいの研究条件、学位、研究テーマ、研究費などの問題について、自由に話し合う、“若者のあつまり”をもちます。若手会員の参加を希望します。くわしくは、東京都立大学生物学教室の岩崎尚彦まで。

4月号大会お知らせ中、原子力研究所見学費用を600円と予告いたしましたが、500円に変更しましたから、お知らせします。

Distribution of Aquatic Phycomycetes in Some Inorganic Acidotrophic Lakes of Japan*

by Shizuo SUZUKI**

Received September 13, 1960

Most Japanese lake waters are almost neutral, their pH value ranging from 6.0 to 7.5¹⁾. However, not a few strongly acidic lakes are found, which belong to organic or inorganic acidotrophic types²⁾. In the former type the acidity originates from the humic substances contained in the lake water, while in the latter it is caused by the inorganic acids derived from the volcanoes.

Since Tanaka's work³⁾ on Lake Onumaike, which belongs to inorganic acidotrophic type, many investigators have studied on the lakes of this type, and many excellent contributions to the floristic and faunal studies are now seen in the literature⁴⁻¹⁰⁾. However, little attention has been paid on the microbial populations in these lakes¹¹⁾. In the present paper the writer deals with the ecology of aquatic Phycomycetes in some inorganic acidotrophic lakes.

Physico-chemical character of inorganic acidotrophic lakes

General features of Lake: In general, the inorganic acidotrophic lakes of Japan are relatively small and shallow. The water color is remarkably blue as in many

Table 1. Chemical analysis of the water in some acidotrophic lakes of Japan.

Lake	Locality	pH	SO ₄ mg./l.	Fe mg./l.	Mn mg./l.	Cl mg./l.	Ca mg./l.
Katanuma	Miyagi	1.9	396	22.3	—	10.4	—
Akadoronuma	Fukushima	2.75	289	278	9.1	—	495
Fudoike	Miyazaki	2.9	35.7	0.62	—	—	—
Akanuma	Fukushima	3.8	324	14.55	1.8	106	166
Sunuma	"	3.8	313	0.46	1.8	8	511
Hyotan-numa	"	4.1	442	0.25	1.9	162	133
Kokenuma	"	4.3	442	0.16	2.6	202	144
Hanarenuma	"	4.3	262	0.12	0.6	8	179
Onumaike	Nagano	4.4	75	0.72	—	—	—
Aodoronuma	Fukushima	4.4	326	0.07	1.7	150	101
Rurinuma	"	4.4	353	0.17	2.2	90	195
Aomiike	"	4.5	322	0.15	—	16	111
Aonuma	"	4.6	260	0.04	1.7	116	181
Benten-numa	"	5.2	364	0.01	1.7	148	186
Bishamon-numa	"	5.2	254	0.36	1.4	96	76
Midoronuma	"	5.8	235	0.76	1.0	182	134

* This report was presented partly at the annual meeting of the Japanese Society of Limnology, held in October 1959, at Toyama University.

** Department of Microbial Chemistry, Faculty of Pharmacy, Tokyo College of Science, Shinjuku, Tokyo.

oligotrophic lakes, but it seems somewhat white. The water of Lake Akadoronuma and that of Akanuma are both reddish and very turbid owing to the suspended iron granules. Most other lakes, however, have a large transparency.

pH: One of the most remarkable characters of these lake waters is the strong acidity caused by the sulfuric or hydrochloric acid from the acidic rivers or springs of volcanoes. The pH value of lake water is 1.9 in Lake Katanuma, 2.75 in Akadoronuma, and 2.9 in Fudoike.

Chemical character: The lake water of this type contains large amounts of mineral substances of about 1-2 g./l.¹²). Especially, sulfate, chlorine and calcium are present in large amounts (Table 1). The content of iron and that of manganese are also very high in comparison with those of harmonic lakes¹⁰); for instance, more than 5 mg./l. are measured in some lakes.

Organic substance in lake bottom: As the lakes of this type are often surrounded by *Phragmites communis* community along the shore, the substrata for the fungi are very abundant. In many cases, the lake bottom is thickly covered with the water mosses, *Leptodictum riparium* var. *longiporium* and *Haprodia* sp.

Distribution of aquatic Phycomycetes

Quantitative feature: In spring, the number of zoospores deviated in the wide range of 0-1800/10 ml. of lake water, and it increased with the decrease of the acidity of lake water (Table 2). The lower productivity of fungi in these lakes is perhaps caused by the strong acidity as well as physico-chemical specificity of the lake water. No saprolegniaceous fungi could be found in strongly disharmonic lakes, such as Lake Katanuma (pH 1.9), Akadoronuma (pH 2.75) and Bishamon-numa (pH 5.2). The disharmonic nature of the former two lakes is caused by the strong acidity of the lake water, while that of the latter by the mineral elements contained in large

Table 2. Amount of zoospore and frequency of aquatic Phycomycetes in the water and the bottom mud of acidotrophic lakes.

	Number of zoospore in lake water (number per 10ml.)	Frequency of fungi in the bottom mud (%)
Katanuma	0	0
Akadoronuma	0	100
Fudoike	21	0
Akanuma	254	70
Sunuma	700	—
Hyotan-numa	188	100
Kokenuma	0	—
Hanarenuma	1020	—
Onumaike	0	71
Aodoronuma	9	—
Rurinuma	90	—
Aomiike	1800	—
Aonuma	2	—
Benten-numa	349	8
Bishamon-numa	0	10
Midoronuma	120	0

amounts (see Table 1). So far as the present investigations are concerned, Lake Fudoike (pH 2.9) is the most acidic lake in which the aquatic Phycomycetes is found.

The aquatic fungi were very scarce or absent in the bottom mud of these lakes. The results obtained are also given in Table 2. The frequency of appearance of fungi was only 0-20 per cent to the total mud samples. This value is far small compared with that either in harmonic or in dystrophic lakes¹³). The bottom muds of Lakes Katanuma and Fudoike are a soft gyttja containing large amounts of deposited sulfur, and that of Lake Midoronuma contains large amounts of iron granules and iron bacteria. No saprolegniaceous fungi could be found in the bottom muds of these lakes. Absence of saprolegniaceous fungi in the former case must be caused by its strong acidity, while in the latter case it may be resulted from other physico-chemical characters of the bottom deposit than pH.

Qualitative feature: With the possible exceptions of certain species, the fungi distributing in acidotrophic lakes seem to be confined to chemical specificity of the lake water. The fungi isolated from the acidotrophic lakes investigated were only five species, in which *Saprolegnia monoica* var. *acidamica* was the preponderant species, and distributed widely in most lakes of this type, while *Achlya flagellata* and *A. racemosa* were relatively scarce. The former of the last two species was found only in Lake Benten-numa and the latter in Lake Midoronuma. The distribution of *Aphanomyces* sp. had a close relation to the pH value of the lake water, and it was limited only in weak acidic lakes.

Table 3. Distributions of aquatic Phycomycetes in the water of acidotrophic lakes.

	<i>Saprolegnia</i> <i>monoica</i> var. <i>acidamica</i>	<i>Achlya</i> <i>flagellata</i>	<i>Achlya</i> <i>racemosa</i>	<i>Aphanomyces</i> sp.	<i>Pythium</i> sp.
Katanuma	—	—	—	—	—
Akadoronuma	—	—	—	—	—
Fudoike	+	—	—	—	—
Akanuma	+	—	—	—	—
Sunuma	+	—	—	—	+
Hytan-numa	+	—	—	—	—
Kokenuma	—	—	—	—	—
Hanarenuma	+	—	—	—	—
Onumaike	—	—	—	—	+
Aodoronuma	+	—	—	—	—
Rurinuma	+	—	—	+	—
Aomiike	+	—	—	—	—
Aonuma	+	—	—	—	—
Benten-numa	+	+	—	+	—
Bishamon-numa	—	—	—	—	—
Midoronuma	+	—	+	+	+

The species number of the fungi found in sample water differed with the pH value of the lake waters. In general, the weak acidic lakes, such as Lake Midoronuma and Benten-numa, were very abundant in species. Namely, four species were found in the former lake and three species in the latter. But *Saprolegnia monoica* var. *acidamica* was only the species found in another acidic lakes. This species dif-

ferred from *S. monoica* in some physiological characters. For instance, it was made clear by culture experiments, that the resistivity of *S. monoica* var. *acidamica* against sulfate is higher than that of the other species. The result will be presented in the near future.

Summary

The distribution of the aquatic Phycomycetes was studied in some inorganic acidotrophic lakes of Japan. The lake waters show strong acidity of pH 1.9–5.8 mainly owing to the dissociation of sulfur compounds. The lake waters contain large amounts of mineral elements, namely, SO_4 : 35.7–442 mg./l., Fe: 0.01–278 mg./l., Mn: 0.6–9.1 mg./l., Cl: 8–202 mg./l., Ca: 76–511 mg./l.

The aquatic Phycomycetes are scarce in these lakes. The number of the zoospores of the fungi is 0–1800/10 ml. in the surface water and increases with the decrease of the pH value of the lake water. The fungi are very scarce or absent in the bottom mud. Five species are obtained from these waters. *Saprolegnia monoica* var. *acidamica* is the commonest species in the water as well as in the bottom mud. The number of the fungus species increases with the decrease of pH value of the lake water.

References

- 1) Yoshimura, S., Sci. Rep. Tokyo Bunrika Daigaku, Sec. C, **2**: 63 (1938).
- 2) ———, Arch. Hydrobiol. **26**: 197 (1933).
- 3) Tanaka, A., Shizen-Kagaku **2**: 181 (1927).
- 4) Masiko, K., Sci. Rep. Tohoku Imp. Univ., Ser. 4, **5**: 331 (1940).
- 5) Negoro, K., Sci. Rep. Tokyo Bunrika Daigaku, Ser. B, **6**: 231 (1944).
- 6) Tamura, T., Jap. Jour. Limn. **2**: 76 (1933).
- 7) Ueno, M., Arch. Hydrobiol. **27**: 571 (1934).
- 8) ———, Jap. Jour. Limn. **8**: 348 (1938).
- 9) ———, Verh. internat. Ver. Limnol. **13**: 217 (1958).
- 10) Yoshimura, S., Negoro, K., and Yamamoto, S., Geogr. Rev. Jap. **12**: 1, 126 (1936).
- 11) Suzuki, S., Jap. Jour. Ecol. **10**: 172 (1960).
- 12) Yoshimura, S., Jour. Assoc. Adv. Sci. Jap. **12**: 13 (1937).
- 13) Suzuki, S., and Hatakeyama, T., Jap. Jour. Limn. **21**: 64 (1960).

摘 要

鈴木静夫： 酸栄養湖における水生菌類の分布と生態

多量の無機塩類を含有している酸栄養型の湖沼の水生菌類を調査し、特に湖水の非調和性の度合と水生菌類の分布との相関について観察した。水生菌類の遊走子数は普通の湖沼に比べてかなり少なく、水の非調和性の強い濁沼 (pH=1.9)、赤泥沼 (pH=2.75)、毘沙門沼 (pH=5.2) には全く水生菌類は見られなかった。棲息する種類もわずかに5種にすぎない。そのうち、*Saprolegnia monoica* var. *acidamica* はほとんどすべての酸栄養湖に見られたが、*Achlya flagellata*, *Achlya racemosa*, *Aphanomyces* sp., *Pythium* sp. は酸性の弱い湖沼にだけ分布していた。(東京理科大学薬学部微生物化学教室)

Concerning the Variants of the *Carex sachalinensis* Group Taxonomic Studies of Cyperaceae 13**

Tetsuo KOYAMA*

Received February 21, 1961

§ 34. Of all species of the genus *Carex* in eastern Asia, *C. sachalinensis* in a wide meaning seems to be taxonomically the most variable in every part of the plant body. Geographically, its quite continuous wide area spreads not only all over Japan but also in central China and southern Saghalien with more than thirteen local variants. Almost all these local populations were originally described by several authors as species, then some of them were reduced to varieties under five species by Ohwi in 1953. But more recently Akiyama again revived the rank of species for most of them. Such diversity in opinions has been chiefly caused by the limited number of material, because these sedges, occurring abundantly in common localities, nevertheless have not been collected sufficiently. This paper gives an evaluation of morphological characters and consequent taxonomic result of my studies made on a large bulk of specimens collected from every part of the area with the kind efforts of several plant collectors, among whom Mr. K. Mayebar, Mr. M. Furuse, Mr. I. Ito and Mr. K. Ogawa are specially worth mentioning. Most of the specimens used for this study are kept in the herbaria of the University of Tokyo (TI), National Science Museum in Tokyo (TNS), Kyoto University (KYO), Hokkaido University (SAP) and Kagoshima University (KAG), while some specimens from China have been checked in the herbaria of the United States National Herbarium (US), Gray Herbarium (GH) and the Arnold Arboretum Herbarium (A) of the Harvard University. My sincere appreciations are therefore also due to the directors and curators of these herbaria.

(1) Morphological observations. The sedges here dealt with are 14 species including *C. alterniflora*, *C. capilliformis*, *C. conicoides*, *C. cuneata*, *C. Duvaliana*, *C. Fernaldiana*, *C. Mayebarana*, *C. pineticola*, *C. pisiformis*, *C. polyschoena*, *C. sachalinensis*, *C. sikokiana*, *C. stenostachys* and *C. tenuinervis*. Their common characters are the variously coloured basal sheaths without elongate blade, ellipsoid utricles tightly enclosing achenes with herbaceous walls and usually loosely disposed in spaced elongate spikes, and tight sheaths of bracts. In the classification of these species, the colour of basal sheaths first caught the investigators' attention as by the purple-red sheaths *C. sikokiana* was separated specifically from its nearest ally, *C. alterniflora*, by both Ohwi and Akiyama. Likewise *C. alterniflora*, *C. alterniflora* var. *aureo-brunnea*, and *C. pisiformis* are characterized by the pale, orange-brown and dark chestnut colours of the outer basal sheaths, respectively. Differing from the so-called trifling colour characters, for instance the white flowered race of the coloured flowered species, the colour of sheaths in this case is regarded to be rather important because of its manysidedness and constancy. In my opinion, a varietal rank can be credited to a variant when it is based on such a colour character of the outer basal sheaths. In the table below are listed these species in accordance with the colour character of sheaths.

I can not agree with the high evaluation of stolons as has hitherto been done. The

* Department of Botany, Faculty of Science, University of Tokyo, Tokyo, Japan.

** Part 12 is in press in *Le Naturaliste Canadien* (1961).

A table showing the colour of basal sheaths

Colour of sheath	Pale	Yellow-brown	Orange-purple
Plant name	<i>C. alterniflora</i>	<i>C. capilliformis</i> <i>C. conicoides</i> <i>C. Duvaliana</i> <i>C. Fernaldiana</i> <i>C. alterniflora</i> var. <i>fulva</i> <i>C. Mayebarana</i> <i>C. sachalinensis</i>	<i>C. alterniflora</i> var. <i>aureo-brunnea</i> <i>C. tenuinervis</i>
Colour of sheath	Fuscous	Red-purple	Dark castaneous
Plant name	<i>C. pineticola</i>	<i>C. sikokiana</i>	<i>C. polyschoena</i> <i>C. pisiiformis</i> <i>C. cuneata</i> <i>C. stenostachys</i>

presence or absence of stolons has been used for making specific segregation, for example *C. cuneata* differs from *C. stenostachys* by the elongate stolons only, so as the relation between *C. alterniflora* and *C. tenuinervis*. The degrees in the density of tufts are quite variable. *C. stenostachys*, which is densely tufted in large clumps as a rule, tends to become looser clumps of several shoots connected with elongate, creeping stolons. Though the latter form was described as *C. cuneata*, the discontinuity between the two became very obscure as I have detected an intermediate form called *C. stenostachys* var. *Ikegamiana*, which makes dense tufts but bears distinct stolons. In this case, the tufts get less dense with the elongation of stolons as the plants extend into the boreal, snowy region of Japan. As to *C. sachalinensis*, however, in the plants from Hokkaido and Saghalien stolons are less developed than in the plants from central Honshu. *C. tenuinervis* differs from *C. alterniflora* var. *aureo-brunnea* only in the absence of stolons. When these two were described, only a few localities were known in Kyushu and Shikoku, but since then many localities have been added also from Kii peninsula and Tokai district. Although it is true that typical *C. tenuinervis* is restricted in Kyushu and Shikoku, while *C. alterniflora* var. *aureo-brunnea* occurs more frequently in Tokai district than in Shikoku and westwards, there are so many transitional forms in Shikoku that I failed to find just where to draw a borderline morphologically. If these two are united into one taxon, the distribution shows that it is a typical western Japanese example. The stolons of these sedges seem to have ecological or geographical significance to some extent, but in most cases they do not give any morphologically definite border. I am accordingly unable to credit supra-varietal status to a plant if it depends upon presence or absence of stolons only.

Differing from such hypogean stolons, *C. conicoides*, *C. pineticola* and *C. alterniflora* var. *arimaensis* bear epigean creepers on which they were based when named. In my observation, the epigean creepers are born apparently in accordance with the ecological conditions of the localities. When the upper part of rhizomes is kept above the soil not sheltered, its new elongation may easily appear in the epigean nature, though it might be hypogean if sheltered. In fact, I have seen several times such conditions in the localities of *C. sachalinensis* near Nikko. Further, a specimen,

Nikko T. Koyama 11044, has both kinds of stolons on the same stock. Like *C. sachalinensis*, *C. pisiformis* also bears both kinds of stolons from the same tuft more commonly. Usually in this latter case, we can see various degrees of stolons from ascending innovation shoots through epigeal stolons to hypogean stolons, occasionally even in the same tuft. The occurrence of epigeal creepers is nothing else than an ecological condition thus has no taxonomic significance. This view has already been suggested by my good senior Dr. M. Mizushima several years ago, but has not been published yet. *C. conicoides* and *C. sachalinensis* are therefore conspecific, as well as *C. alterniflora* var. *arimaensis* is synonymous with *C. alterniflora* itself.

C. alterniflora has been separated from *C. sachalinensis* in the relative length of spikes to their bracts, namely in the former rather long blades of bracts conspicuously exceed the spikes on enclosed short peduncles, whereas in the latter the spikes on longly exerted peduncles invariably overtop their bracts. This dimensional difference is well correlated with the leaf characters that the blades are softer and unexceptionally more blunt-tipped in *C. sachalinensis* than in *C. alterniflora*. This character is reliable so far as I have seen specimens.

The characters belonging to utricles have seldom been discussed except that *C. sachalinensis* var. *longiuscula* and *C. alterniflora* var. *elongatula* were separated from their mother species in their elongated beaks of utricles. The thickness of utricular walls together with the associated hair character fairly well separates the *C. pisiformis* and its close allies from the *C. sachalinensis* group. In *C. pisiformis* with its close allies including *C. polyschoena*, *C. stenostachys* and *C. cuneata*, the utricular walls are thick herbaceous with strongly elevated veins. The hairs on them are constantly dense. In the other sedges including *C. sachalinensis*, *C. alterniflora*, etc., on the other hand, the utricular walls are thick membraneous to thin herbaceous with hardly elevated faint veins usually. The hairs on them are, if present, of pubescence of varying degrees. When the colour of the outer basal sheaths is used for distinguishing *C. pisiformis* from *C. alterniflora* complex, it becomes often difficult to separate the former from *C. alterniflora* var. *aureo-brunnea*, but the two entities in problem are to be coordinated in the same taxonomic rank

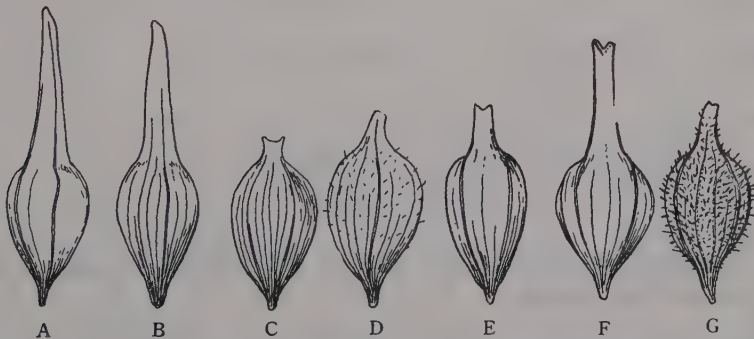


Fig. 34. Utricles of *C. sachalinensis* group. A. *C. Mayebarana* Ohwi (= *C. pisiformis* Boott var. *Mayebarana* T. Koyama). B. *C. sachalinensis* F. Schmidt var. *longiuscula* Ohwi (= *C. p.* var. *longiuscula* T. Koyama). C. *C. sachalinensis* F. Schmidt (= *C. p.* var. *sachalinensis* Kükenth.). D. *C. sachalinensis* F. Schm. var. *iwakiana* Ohwi (= *C. p.* var. *sachalinensis* Kükenth. forma *iwakiana* T. Koyama). E. *C. alterniflora* Franch. (= *C. p.* var. *alterniflora* T. Koyama). F. *C. alterniflora* Franch. var. *elongatula* Ohwi (*C. p.* var. *elongatula* T. Koyama). G. *C. pisiformis* Boott. All $\times 6$. (Icon. orig.)

on the basis of these utricular characters.

The utricles with thinner walls are mostly glabrous as in *C. sachalinensis*, *C. alterniflora* and *C. Fernaldiana*, but each differentiates a race with pubescent utricles. They are *C. sachalinensis* var. *iwakiana*, *C. alterniflora* var. *musashiensis* and *C. capilliformis*, which I am treating as the forms of the three species mentioned above. It is of interest that the variants with long-beaked utricles constantly grow in the *Fagus* belt never coming down to the Chestnut belt below it. Another similar occasion is observed in var. *lanceata* of *C. oxyandra*. The variety, bearing the utricles with a long beak, occurs in subalpine regions, while its mother species with less elongated beaks of utricles does not reach such a high altitude. These long-beaked races can be maintained as being varieties for the reasons that these populations depend on the zonal isolation and that no intermediate form has been found as to the length of beaks at least.

(2) Cytological observation. The chromosomes of the six related species were reported by Prof. N. Tanaka (1940¹), 1948²)) as follows:

<i>C. stenostachys</i>	$2n=56$
<i>C. alterniflora</i>	$2n=60, 68, 76, 78, 84$
<i>C. Duvaliana</i>	$2n=75, 76, 78$
<i>C. Fernaldiana</i>	$2n=66, 67, 68, 72$
<i>C. sachalinensis</i>	$2n=56, 62, 64, 66, 68$
<i>C. sikokiana</i>	$2n=60$

The data, however, need a few modification in the taxonomic respect. The specimen from Kamikochi, central Japan, on which $2n=56$ of *C. sachalinensis* is based, is in my determination *C. cuneata*, and the data should be transferred to *C. stenostachys*. $2n=60$ of *C. sikokiana* was examined in a specimen from Mt. Amagisan, where such a western Japanese species does not occur, and it must be $2n=60$ of *C. alterniflora*.

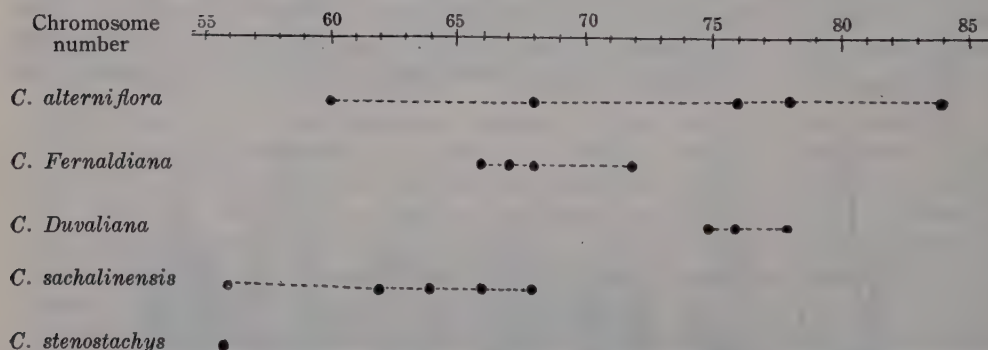


Fig. 35. Chromosome numbers of the 5 examined species of the *C. sachalinensis* group. For the interpretation see the text.

These revised data are plotted in Fig. 35 for use in taxonomy. All but *C. stenostachys* are represented in aneuploid series of various ranges. An important matter is that the narrow ranges of *C. sachalinensis*, *C. Fernaldiana* and *C. Duvaliana* completely fall under the very wide range of *C. alterniflora*. This manifestly coincides with the fact that, as compared with *C. alterniflora* each of these first three sedges have more definite taxonomic characters of its own, among which is the wholly tomentose vegetative parts of *C. Duvaliana*, the capillary leaves of *C. Fernaldiana*, or elongated peduncles of *C. sachalinensis*. And at the same time, it is pos-

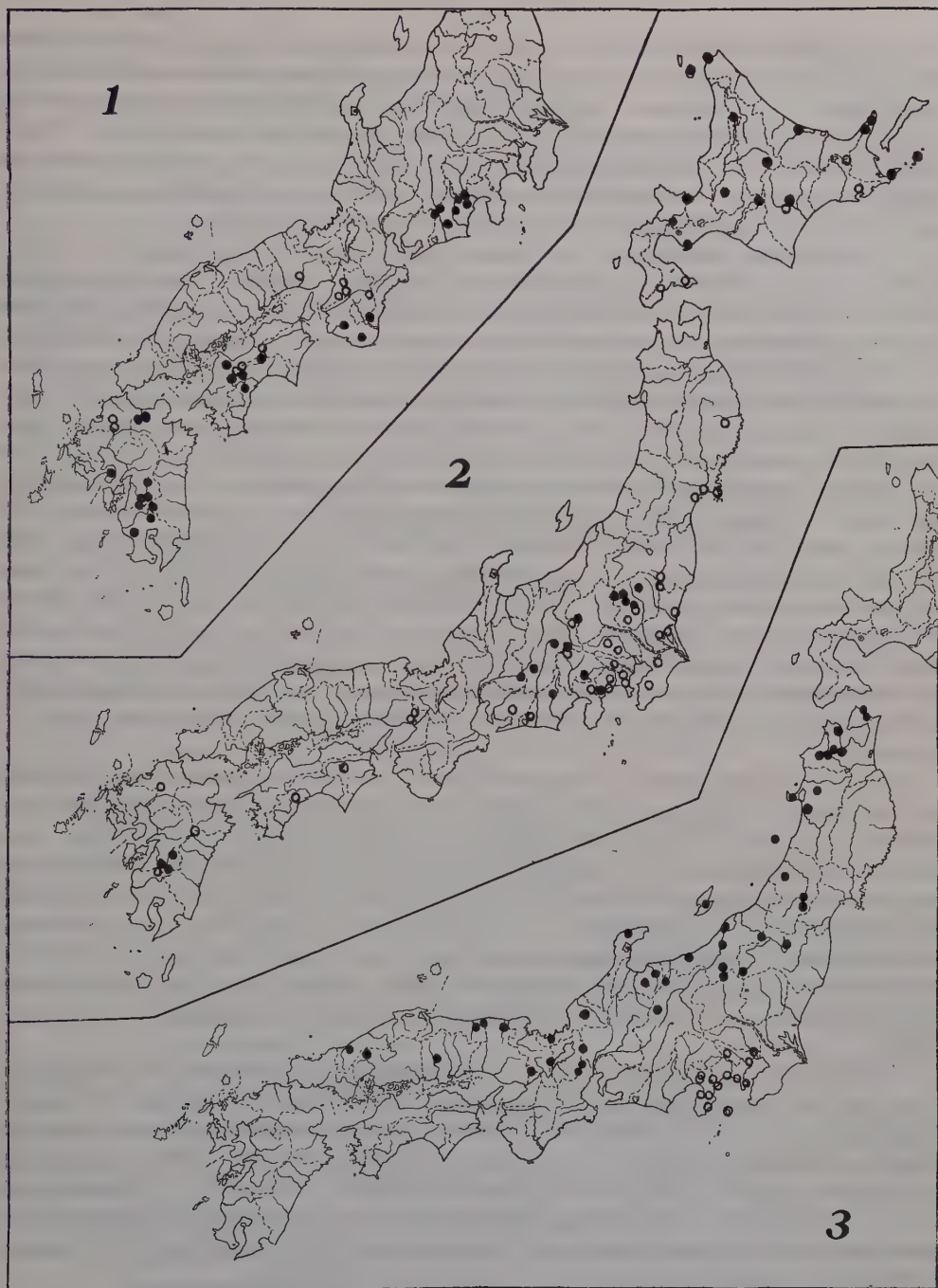


Fig. 36. Distribution of the sedges of *C. sachalinensis* group.

1. Dot: *C. pisiiformis* Boott var. *aureo-brunnea* T. Koyama (= *C. alterniflora* Franch. var. *aureo-brunnea* Ohwi; *C. tenuinervis* Ohwi). Circle: *C. p.* var. *sikokiana* T. Koyama (= *C. sikokiana* Franch. and Savat.).

2. Dot: *C. p.* var. *sachalinensis* T. Koyama (= *C. sachalinensis* F. Schmidt; *C. conicoides* Honda). Circle: *C. p.* var. *alterniflora* T. Koyama (= *C. alterniflora* Franch.; *C. pseudostrigosa* Lévl. and Vant.).

3. Circle: *C. pisiiformis* Boott var. *pisiiformis*. Dot: *C. p.* var. *cuneata* T. Koyama (= *C. cuneata* Ohwi; *C. stenostachys* Franch. and Savat.).

sible to consider that the variants of the *C. alterniflora-sachalinensis* complex would have been differentiated from a common ancestor presumably allied to *C. alterniflora* respectively as a chromosomal population thereof. It is also of interest taxonomically that *C. stenostachys* is cytologically more distinct than any other variant. Being well correlated to the utricular characters mentioned above, this cytological evidence shows that among all variants the discontinuity between the *C. pisiformis* group and the *C. sachalinensis-alterniflora* complex is getting more conspicuous.

(3) Geographic distribution. As illustrated in Fig. 36, the variants of *C. sachalinensis* in a wide meaning is clearly divided into two classes on the ditributional ground, i.e. the plants distributed on the Japan Sea side of the mainland of Japan and those distributed on the Pacific side in the mainland of Japan. *C. stenostachys* and *C. cuneata* belong to the former category (Fig. 36, 3). These two have been separated by the presence of creeping stolons in the latter, but as was already suggested the stoloniferous habit in *C. cuneata* is nothing else than an ecological condition perhaps caused by the deep snow in the Japan Sea side of northern Japan. This view is also supported by the following reasons. The specimens from the northernmost part of Kwanto district, though geographically they are determinable as *C. cuneata*, tend to lack the elongate stolons and to bear utricles rather loosely thus they can hardly be separable from *C. stenostachys*. Some specimens in similar state were also collected at the foot of Mt. Shirouma by Mr. Furuse. Secondly, in the plants of *C. cuneata* from Hokuriku district, leaves and culms are more robust bearing densely flowered thick spikelets, however, this is also an ecological condition and such robustness can be seen quite commonly also in *C. multifolia*, *C. belpharicarpa*, etc. I, at last, came to a conclusion that *C. cuneata* can not be separated from *C. stenostachys*.

All variants other than *C. stenostachys* and *C. cuneata* are scattered on the Pacific side of Japan. They are further subdivided into about three types of distribution. As seen in Fig. 36-3, *C. pisiformis* occurs in a small region including Mt. Fuji, Hakone, Idzu peninsula and northern Idzu islands, and falls under the distribution type IV-A of Mr. Kanai (1958³). From this geographical difference, *C. pisiformis* can be better treated to be coordinated to *C. stenostachys* of Japan Sea side at the same taxonomic rank, though these two are strikingly akin. The accompanying morphological character is only the colour of the outer basal sheaths which are dark chestnut to orange-brown in *C. stenostachys* while light chestnut to orange-brown in *C. pisiformis*.

C. alterniflora var. *aureo-brunnea*, *C. Duvaliana* and *C. sikokiana* are distributed in central and western Japan only, falling under the distribution type III of Mr. Kanai. *C. alterniflora* var. *aureo-brunnea* of my sense includes a non-stoloniferous population called *C. tenuinervis* up till now. The reason of this union was already discussed in the preceding chapter. The other variants including *C. alterniflora*, *C. sachalinensis* and *C. Fernaldiana* occur throughout the Pacific side from Kyushu to Hokkaido as shown in Fig. 36-2. The collection of *C. sachalinensis* in Northeast district of Honshu is far from complete. Perhaps it will not be absent from there.

(4) Taxonomic conclusion.

1. No taxonomic character was found to credit a specific rank to any of the variants here treated.

2. The colour character of outer basal sheaths, the relative length of bract blades to their spikes, and utricular characters morphologically delimit varieties equivalently. This view is also supported by both cytological and geographic points.

of view.

3. Presence of creeping stolons can not be reliable in delimitation of taxa. They are only an ecological condition in most cases.

4. As to the nomenclature of related species, several specialists including Franchet and Kükenthal have applied the Boott's name, *C. tenuissima*, to a plant commonly known as *C. Fernaldiana*. However, this does not hold good, because if *C. tenuissima* be *C. Fernaldiana*, "It is hardly conceivable that Bunge should have marked this '*C. panicea*, L.' and that Boott should have described it as '*C. panicea*, L.', affinis." (from C. B. Clarke in Journ. Linn. Soc. 36: 313-4 (1904)). It is quite regrettable that the type of *C. tenuissima* has never been seen by all authors concerned and I also failed to do that. It may possibly be in Leningard together with other Bunge collections from northern China. Accordingly the earliest correct name of *C. sachalinensis* should be *C. pisiformis*, when the sedges here dealt with are united into one species as I do.

A KEY TO THE VARIANTS OF *Carex pisiformis*

1. Utricles densely hirsute, rather thick herbaceous, veins strongly elevated.
2. Floral scales chestnut brown.
 3. Basal sheaths and scales of stolons orange-brown; spikes loosely flowered. 1. var. *pisiformis*.
 3. Basal sheaths and scales of stolons (if present) chestnut brown or fuscous; spikes loosely to densely flowered. 2. var. *cuneata*.
2. Floral scales pale-green; rhizome never stoloniferous. 3. var. *koreana*.
1. Utricles as a rule glabrous, when hairy loosely pubescent, membranous, faintly veined.
4. Bract blades longer than spikes thereof; apices of blades acute.
5. Vegetative parts glabrous.
6. Basal sheaths orange-brown, yellow-brown or pale.
 7. Basal sheaths and scales of stolons orange- or yellow-brown.
 8. Beak of utricles shorter than the body.
 9. Leaves filiform, 1.5 mm wide or less.
 10. Floral scales at least staminate ones fulvous; leaves and culms soft. 7. var. *fulva*.
 10. Floral scales pale; leaves and culms rigid. 5. var. *aureo-brunnea*.
 8. Beak of utricles as long as or longer than the body. 6. var. *elongatula*.
 7. Basal sheaths and scales of stolons pale. 11. var. *alterniflora*.
 11. Utricles quite glabrous. f. *alterniflora*.
 11. Utricles sparsely pubescent. f. *musashiensis*.
 6. Basal sheaths purple-brown, or purple-red.
 12. Utricles glabrous; leaves usually more than 2.5 mm wide; basal sheaths purple-red. 10. var. *sikokiana*.
 12. Utricles hispidulous or sparsely so; leaves 0.5-2.5 mm wide; basal sheaths purple-brown. 4. var. *major*.
5. Vegetative parts, at least leaf sheaths, pubescent.
 13. Utricles several-veined, beak shorter than the body; vegetative parts wholly densely pubescent. 9. var. *Duvaliana*.
 13. Utricles 2-veined, beak longer than the body; leaf sheaths minutely puberulent. 12. var. *Mayebarana*.
4. Spikes overtopping bract blades; apices of blades relatively suddenly acute.
 14. Floral scales brownish; spikes dense-flowered. 13. var. *pineticola*.
 14. Floral scales pale; spikes loosely flowered.
 15. Beak of utricles shorter than the body. 14. var. *sachalinensis*.

16. Utricles quite glabrous.f. *sachalinensis*.
 16. Utricles puberulent.f. *iwakiana*.
 15. Beak of utricles as long as long or longer than the body.15. var. *longiuscula*.

NOMENCLATORIAL TREATMENTS*

Carex (Praecoces) **pisiformis** Boott in A. Gray, Narr. Exped. Perry 2: 324 (1857), sensu emend. (vid. typ.).

1. var. **pisiformis**.—*C. amphora* Franch. & Savat., Enum. Pl. Japon. 2: 142 (1877) & 566 (1879). (vid. sp. auth.). Japanese name: *Hommonji-suge*. Distribution: See Fig. 36.

2. var. **cuneata** (Ohwi) T. Koyama, comb. nova et sensu emend.

C. stenostachys Franch. & Savat., Enum. Pl. Japon. 2: 142 (1877) & 569 (1879), syn. nov.—*C. cuneata* Ohwi in Mem. Coll. Sci. Kyoto Imp. Univ. B, 6: 256 (1931)—*C. stenostachys* Fr. & Sav. var. *cuneata* (Ohwi) Ohwi & T. Koyama ex T. Koyama in Act. Phytotax. Geobot. 16: 10 (1955)—*C. stenostachys* Fr. & Sav. var. *Ikegamiana* T. Koyama l. c. 11 (1955), syn. nov. Japanese name: *Nishino-hommonjisuge*, *Michi-noku-hommonjisuge*. Distribution: See Fig. 36.

3. var. **koreana** (Nakai) T. Koyama, comb. nova e typo.

C. polyschoena Lévl. & Vant. ex Lévl. in Bull. Acad. Intern. Géogr. Bot. 12: 9 (1903)—*C. albomas* C. B. Clarke in Journ. Linn. Soc. 36: 270 (1903)—*C. pisiformis* Boott forma *polyschoena* (Lévl. & Vant.) Kükenth., Cyper-Caric. 477 (1909)—*C. indistinctum* Lévl. & Vant. in Fedde, Repert. Sp. Nov. 5: 194 (1908)—*C. umbrosa* Host var. *koreana* Nakai in Bot. Mag. Tokyo 28: 327 (1914). Distribution: Is. Tsushima, Korea, Manchuria.

4. var. **major** (Kükenth.) T. Koyama, comb. nova.

C. capilliformis Franch. in Bull. Soc. Philom. Paris, 8^e sér., 7: 89 (1895). (vid. sp. auth.).—*C. capilliformis* Franch. var. *major* Kükenth. in Engl., Bot. Jahrb. 36, Beibl. Nr. 82, 9 (1905). Distribution: Central China.

5. var. **aureo-brunnea** (Ohwi) T. Koyama, comb. nova e typo.

C. tenuinervis Ohwi in Mem. Coll. Sci. Kyoto Imp. Univ. B, 5: 266 (1930), syn. nov. e typo—*C. alterniflora* Franch. var. *aureo-brunnea* & *tenuinervis* Ohwi l. c. 6: 259 (1931)—*C. sachalinensis* F. Schmidt var. *aureo-brunnea* (Ohwi) Ohwi in Bull. National Sci. Mus. No. 33, 67 (1953)—*C. sachalinensis* F. Schmidt var. *tenuinervis* (Ohwi) T. Koyama in Bull. Arts & Sci. Div., Ryukyu Univ. No. 3, 72 (1959). Japanese name: *Cha-itosuge*, *Tsurunashi-oh-itosuge*. Distribution: Honshu, on Pacific side, Fuji-gawa river and westwards; Shikoku; Kyushu.

6. var. **elongatula** (Ohwi) T. Koyama, comb. nova e typo.

C. alterniflora Franch. var. *elongatula* Ohwi in Mem. Coll. Sci. Kyoto Imp. Univ. B, 6: 260 (1931)—*C. sachalinensis* F. Schm. var. *elongatula* (Ohwi) Ohwi in Bull. National Sci. Mus. No. 33, 68 (1953). Japanese name: *Kuju-suge*. Distribution: Northern Kyushu (in upper *Fagus* zone only).

7. var. **fulva** (Ohwi) T. Koyama, comb. nova e typo.

C. artinux C. B. Clarke in Kew Bull. Add. Ser. 8, 81 (1908)—*C. alterniflora* Franch. var. *fulva* Ohwi in Mem. Coll. Sci. Kyoto Imp. Univ. B, 11: 367 (1936)—*C. sachalinensis*

* In this paper are cited only important synonyms including basionyms. The detailed synonymy will be given in my monograph of East Asiatic Cyperaceae, Pt. II.

sis F. Schm. var. *fulva* (Ohwi) Ohwi in Bull. National Sci. Mus. No. 33, 68 (1953). Japanese name: *Ki-itosuge*. Distribution: Mt. Hakusan and n.-e.-wards on Japan Sea side of Honshu, in subalpine zone.

8. var. **Fernaldiana** (Lévl. & Vant.) T. Koyama, comb. nova.

‘*C. tenuissima* Boott’: Franch. & Savat., Enum. Pl. 2: 147 (1877) & auct. plur.—*C. Fernaldiana* Lévl. & Vant. in Bull. Acad. Intern. Géogr. Bot. 10: 276 (1901). (vid. sp. auth.)—*C. Mariesii* & *ischne* C. B. Clarke in Kew Bull. Add. Ser. 8, 80 (1908)—*C. sachalinensis* F. Schmidt var. *Fernaldiana* (Lévl. & Vant.) T. Koyama in Bull. Arts & Sci. Div., Ryukyu Univ. No. 3, 72 (1959). Japanese name: *Ito-suge*. Distribution: Japan, Korea, Formosa.

9. var. **Duvaliana** (Franch. & Savat.) T. Koyama, comb. nova.

C. Duvaliana Franch. & Savat., Enum. Pl. Japon. 2: 568 (1879) (vid. sp. auth.)—*C. Hilgendorffiana* Böckl. in Engl., Bot. Jahrb. 5: 518 (1884)—*C. hololasius* Lévl. & Vant. in Bull. Acad. Intern. Géogr. Bot. 10: 280 (1901)—*C. tenuissima* Boott var. *Duvaliana* (Franch. & Savat.) Kükenth., Cyper.-Caric. 476 (1909)—*C. sachalinensis* F. Schm. var. *Duvaliana* (Franch. & Savat.) T. Koyama l. c. 72 (1959). Japanese name: *Ke-suge*. Distribution: Hokkaido, Honshu.

10. var. **sikokiana** (Franch. & Savat.) T. Koyama, comb. nova.

C. sikokiana Franch. & Savat., Enum. Pl. Japon. 2: 573 (1879)—*C. tenuissima* Boott var. *sikokiana* (Fr. & Sav.) Kükenth., Cyper.-Caric. 475 (1909)—*C. alterniflora* Franch. var. *arimaensis* Ohwi in Mem. Coll. Sci. Kyoto Imp. Univ. B, 6: 259 (1931), syn. nov. e typo.—*C. sachalinensis* F. Schm. var. *sikokiana* (Fr. & Sav.) & *arimaensis* (Ohwi) Ohwi in Bull. National Sci. Mus. No. 33, 67 (1953). Japanese name: *Beni-itosuge*, *Kwansai-oh-itosuge*. Distribution: Kinki and western districts of Honshu, Shikoku.

11. var. **alterniflora** (Franch.) T. Koyama, comb. nova.

C. alterniflora Franch. in Bull. Soc. Philom. Paris, 8^e sér., 7: 51 (1895)—*C. pseudostrigosa* Lévl. & Vant. in Bull. Acad. Intern. Géogr. Bot. 11: 109 (1902)—*C. Duvaliana* Franch. & Sav. var. *alterniflora* (Franch.) Kükenth. ex Matsum., Ind. Pl. Japon. 2 (1): 108 (1905)—*C. scabro-aristata* Akiyama in Journ. Fac. Sci. Hokkaido Imp. Univ. 5, 1: 58, t. 3 (1931)—*C. sachalinensis* F. Schm. var. *alterniflora* (Franch.) Ohwi in Bull. National Sci. Mus. No. 33, 67 (1953)—*C. alterniflora* Franch. var. *pseudostrigosa* (Lévl. & Vant.) Akiyama, Caric. Far East. Reg. As. 197 t. 201 (1955). Japanese name: *Oh-itosuge*. Distribution: See Fig. 36.

11-b. forma **musashiensis** (Hiyama) T. Koyama, stat. nov.

C. sachalinensis F. Schm. var. *musashiensis* Hiyama in Journ. Jap. Bot. 29: 160 (1954). Distribution: Kwanto district.

12. var. **Mayebarana** (Ohwi) T. Koyama, stat. nov. e typo.

C. Mayebarana Ohwi in Mem. Coll. Sci. Kyoto Imp. Univ. B, 5: 256 (1930). Japanese name: *Ke-hiesuge*. Distribution: Central Kyushu, in upper *Fagus* zone.

13. var. **pineticola** (Ohwi) T. Koyama, comb. nova e typo.

C. pineticola Ohwi in Bull. National Sci. Mus. No. 26, 5 (1949)—*C. sachalinensis* F. Schm. var. *pineticola* Ohwi l. c. No. 33, 68 (1953)—*C. sachalinensis* F. Schm. var. *pineticola* Ohwi forma *calvescens* Hiyama in Journ. Jap. Bot. 29: 395 (1954), syn. nov. Japanese name: *Matsukaze-suge*. Distribution: Kwanto district, near Inubo Cape.

14. var. **sachalinensis** (F. Schmidt) Kükenth. ex Matsumura, Index Pl. Japon. 2 (1): 126 (1905).

C. sachalinensis F. Schmidt, Reisen Amurl. u. Ins. Sachal. 194, t. 6: ff. 14-17

(1868)—*C. pseudo-conica* Franch. & Savat., Enum. Pl. Japon. 2: 144 (1877) & 570 (1879), pro maxim. parte incl. lectotypum—*C. korsakoviensis* Léveillé in Bull. Acad. Intern. Géogr. Bot. 19: 34 (1909)—*C. conicoides* Honda in Bot. Mag. Tokyo 42: 506 (1928), syn. nov. e typo—*C. sachalinensis* F. Schm. var. *conicoides* (Honda) Ohwi in Bull. National Sci. Mus. No. 33, 68 (1953). Japanese name: *Gongen-suge*. Distribution: Saghalien, Kuriles (Is. Yotorofu and s.-w.-wards), Hokkaido, Honshu (upper *Fagus* zone only).

14-b. forma **iwakiana** (Ohwi) T. Koyama, stat. nov. e typo.

C. sachalinensis F. Schm. var. *iwakiana* Ohwi in Mem. Coll. Sci. Kyoto Imp. Univ. B, 11: 368 (1936), in descriptione. Distribution: Northern Kwanto district.

15. var. **longiuscula** (Ohwi) T. Koyama, comb. nova e typo.

C. niko-montana Akiyama in Bot. Mag. Tokyo 45: 472 (1931)—*C. sachalinensis* F. Schmidt var. *longiuscula* Ohwi in Mem. Coll. Sci. Kyoto Imp. Univ. B, 11: 368 (1936), in descr. Japanese name: *Miyama-aosuge*. Distribution: Kwanto and central districts of Honshu, upper *Fagus* zone.

References

- 1) Tanaka, N., Japan. Journ. Bot. 11: 215 (1940).
- 2) —, The Problem of Aneuploidy (in Japanese), 327 pp. and 38 pls., Tokyo (1948).
- 3) Hara, H., and Kanai, H., Distribution Maps of Flowering Plants in Japan, Fasc. 1, 1-14 (1958).

摘 要

小山鐵夫： ホンモンジスゲ・ゴンゲンスゲ群の変異について
(カヤツリグサ科の分類学的研究 13)

広義のゴンゲンスゲはスゲ類中最も変化の多い種類で、見方によっては13種以上に細分されることもある。しかし今までの研究は局部的に限られた標本で行なわれていたきらいがあり、したがって生態的な変異にも分類群としての階級が与えられたものも少ない。この研究ではすべての形態的特徴を再検討し、細胞学のおよび、日本全土から集めた分布上のデータをもあわせて、ゴンゲンスゲの変異を整理し、さらにホンモンジスゲとその類縁も結局ゴンゲンスゲの変異のうちに含まれるべきことをのべた。(東京大学理学部植物学教室)

Productivity in Sessile Algal Community of Japanese Mountain River

by Hiromu KOBAYASI*

Received February 24, 1961

In a previous study¹⁾, the standing crop of sessile algal community in the mountain section of the River Arakawa was measured in chlorophyll amount and habitat factors determining the standing crop were investigated.

In order to obtain the more precise information on the primary production in the mountain river, the detailed investigation should be made concerning the photosynthesis of sessile algae and its relation to the habitat factors.

As an indirect method to measure the primary production, so-called chlorophyll method has recently been employed by several investigators. This method is excellent in analysing the relationship between the primary production and habitat factors. In the present study, therefore, the primary production of the mountain section of the River Arakawa was pursued by the chlorophyll method.

Habitat Conditions on the River Bed

1. Nutrient elements in river water.

According to the data presented by Hayakawa (see H. Kobayasi¹⁾) the locational changes in the chemical components in the water could not be found at least within the mountain section of the River Arakawa. The amount of the nutrient elements such as nitrogen and phosphate was roughly the same through the watercourse. The



Fig. 1. Photograph of the celestial hemisphere taken at Station 6.

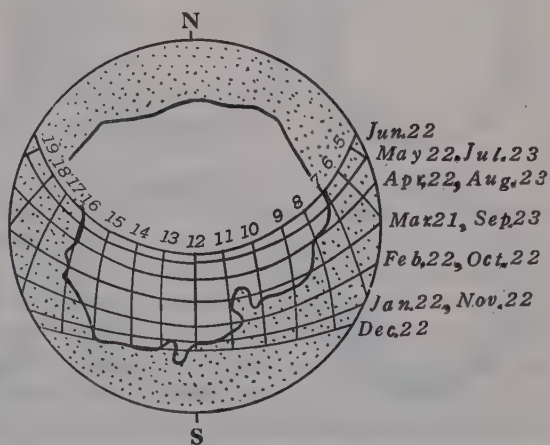


Fig. 2. Interpreted tracing of Station 6 as recorded on photograph of the celestial hemisphere, on which the details are also indicated when the transparent overlay of sun course at latitude 35° is placed over.

* Botanical Institute, Faculty of Science, Tokyo University of Education, Otsuka, Tokyo.

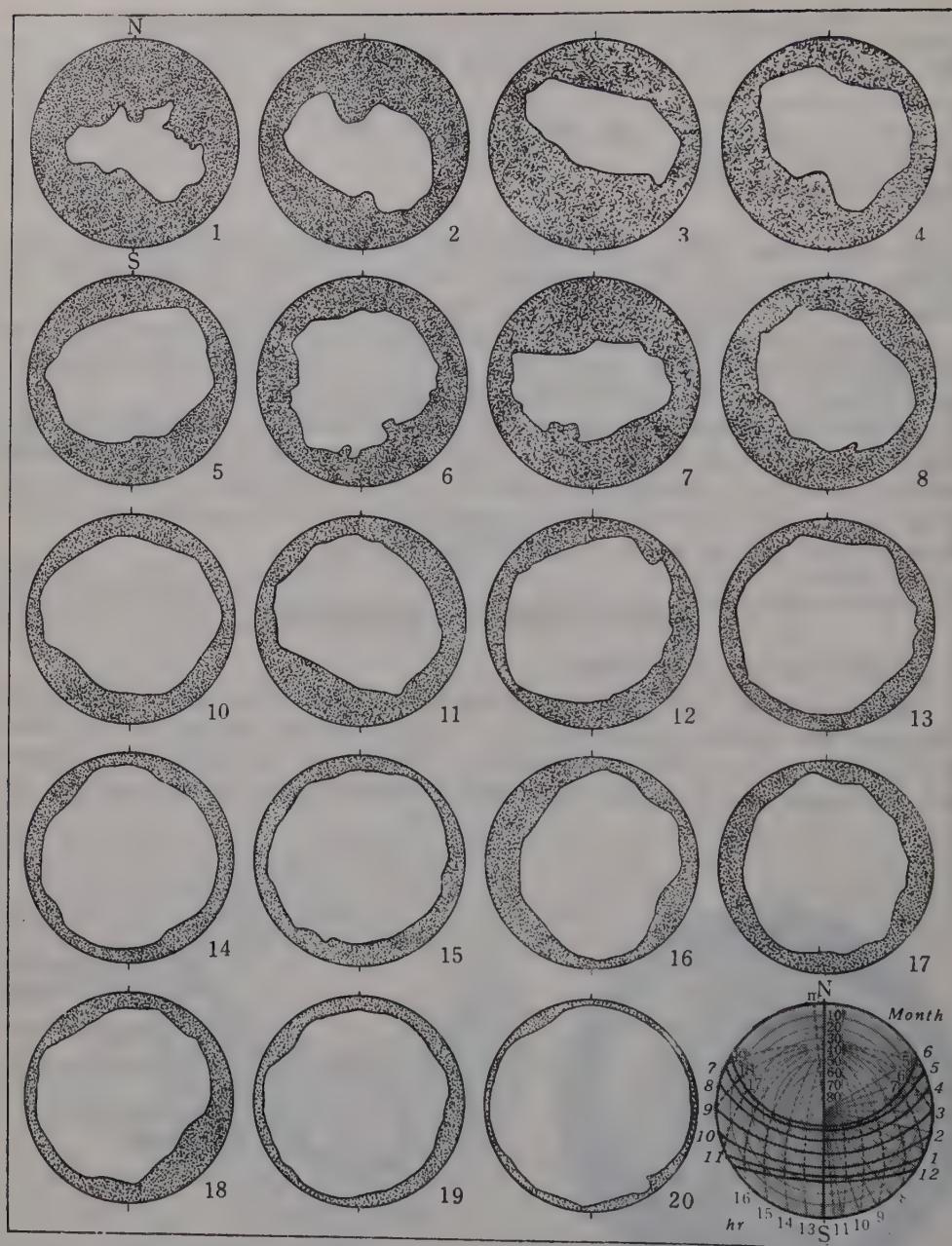


Fig. 3. Interpreted tracing of photographs of the celestial hemisphere at various stations along the watercourse in the mountain section of the River Arakawa (Station 1-20) and transparent overlay of sun course at latitude 35° . N and n indicate the true north and the magnetic north, respectively.

values of 0.05 ppm. in phosphate and 0.3 ppm. in nitrogen correspond to those in the eutrophic lakes in Japan.

As indicated in a previous paper¹⁾, the water temperature increased successively

with the distance from the source of the river. Difference of the water temperature between the canyon section and lower section was about 5° throughout the year.

2. Light condition.

For the study of the primary production of the mountain river, it is indispensable to make the light condition clear. The light condition on the bottom of the mountain river varied conspicuously from place to place with the difference of the shade formed by the mountains and trees standing along the river. For this reason, the light intensity and duration of insolation on the river bed were determined with the photograph of the celestial hemisphere after Monsi and Saeki³⁾. Figs. 1, 2 show the photograph taken at Station 6. Fig. 3 summarizes the interpreted tracing of photographs taken at various stations along the watercourse of the mountain section. White portion on the figures is the open area and dotted one is the occluded area of the sky. As seen in Fig. 4, the open sky increases with the distance from the source of the river. The open sky is 20% to 50% of the whole celestial hemisphere in the canyon section, and 60% to 80% in the lower section. The intensity of the diffused light falling on the river bed can be deduced indirectly from the ratio of the open sky area to the whole celestial hemisphere. The diffused light intensity from blue sky is on daily average 10 klux, whereby the station having the open sky of s area may receive the diffused light of $10 \cdot s/S$ klux, where S being the area of the whole celestial hemisphere.

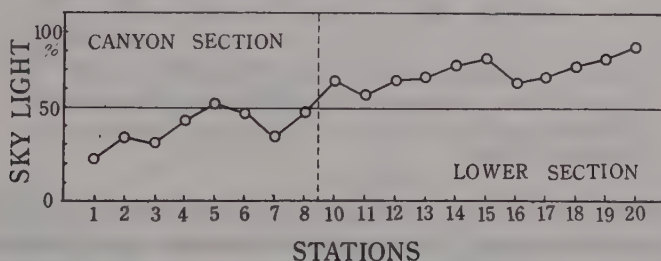


Fig. 4. Locational change in open sky at various stations in the mountain section of the River Arakawa.

As made by Monsi and Oshima²⁾, and McConnel and Sigler⁴⁾, the intercepted insolation on the bottom of the river at any time of a year is determined by reading of transparent overlay of sun course at latitude 35° , which was taken from Hirayama's data⁵⁾. As can be seen in Fig. 2, the sunshine duration on the river bed corresponds to the hours on the sun course within the area of the open sky on the photograph. By means of the foregoing overlay, it can be illustrated clearly that the sunshine duration on the river bed varies conspicuously through a year and with the shape of the open sky. For example, the area of the open sky at Stations 3 and 7 is 30% and 34%, while the sunshine duration in summer is about 6.5 hrs. at the former and 10.5 hrs. at the latter station, i.e., relatively the open sky is 100:113 and the sunshine duration is 100:162. From these conspicuously different results, it can be surmised that the light condition of the river bed is one of the important habitat factors determining the primary production of the mountain section of the River Arakawa.

Photosynthetic Activity of Sessile Algae

The photosynthesis was measured by the well known light and dark bottles method

using Winkler's method. The sessile algae were removed from the river bed, homogenized, and suspended in the bottles filled with the river water. Since the materials at Station 17 were characterized in summer with *Cladophora*, in this case the algal fragments of 0.5–1.0g. fresh weight were sealed in each bottle. The bottles were laid on the river bed, from which the materials were taken and then left 2 hours in the midday. For one series experiment, 6 groups of every 3 bottles were prepared and each group of them was exposed under the light of 5%, 10%, 20%, 50% and 100% of the full sun light. The gradation of the light intensity was controlled with a graded series of neutral vinyl chloride cloths. During the exposing duration, the light intensity on the surface of the river was measured with the photo-electric cell. It was found that the light intensity continues the constant state at least during the midday. The respiration was measured in the bottles covered with a black vinyl chloride cloths. The photosynthesis was expressed by the amount of oxygen evolved

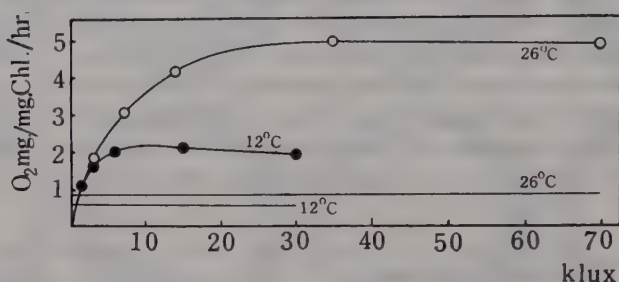


Fig. 5. Typical photosynthesis-light curves obtained in summer (Open circles, Jul. 15, 1960) and winter samples (Filled circles, Nov. 20, 1960). Simple solid lines show the respiration values.

per mg. chlorophyll. Fig. 5 indicates the typical photosynthesis-light curves obtained in summer and winter samples. The materials in summer were *Cladophora* fragments and those in winter were diatoms and blue-green algae. The pattern of the photosynthesis-light curve in the former indicated the sun-type with 4.9 mg. O_2 /mg.chl./hr. at the light saturation point and the shade-type with 2.1 mg. O_2 /mg.chl./hr. in winter. According to Ichimura⁶), the maximum photosynthetic activity of phytoplankton in Japanese lakes is 8–10 mg. O_2 /mg.chl./hr. in the samples taken from the eutrophic lakes and 4–5 mg. O_2 /mg.chl./hr. in the mesotrophic lakes. In the oligotrophic lakes, it is 1.0–2.5 mg. O_2 /mg.chl./hr. Therefore, the photosynthetic activity of sessile algae in the mountain river can be compared with those of the mesotrophic lakes. As already been noticed by McConnel and Sigler⁴), it is probable that the suppression of photosynthesis may occur on such a confined algae in the bottles or jars. Therefore, the foregoing values obtained in the present study seems to be much lower than the true ones *in situ*. However, it is noticeable that the extremely low rates of 0.5 to 0.7 mg. O_2 /mg.chl./hr. were reported by McConnel and Sigler⁴) with the sessile algae of the River Logan, Utah.

Estimation of the Productivity of Sessile Algae from Chlorophyll and Light Data

Using the procedure provided by Ryther⁷), the daily change of the photosynthetic rate of sessile algae on the river bed can be deduced indirectly from the photosynthesis-light curve and the daily incident radiation data. Fig. 7 shows the daily change

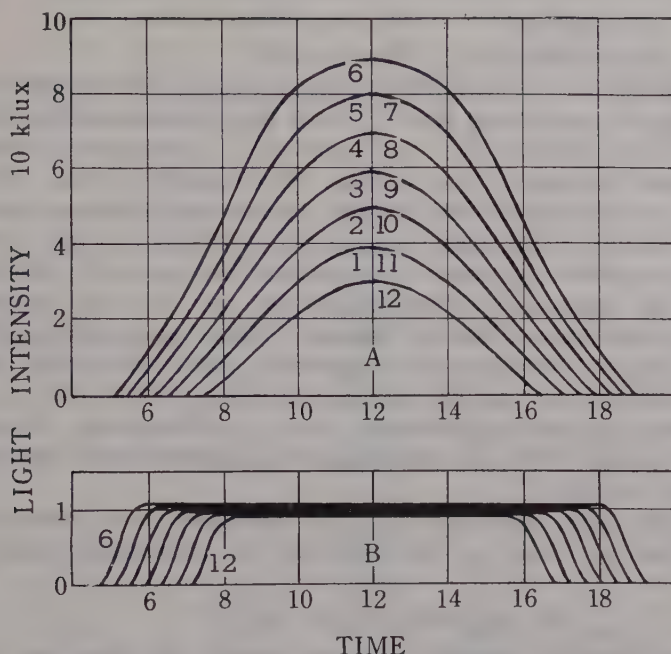


Fig. 6. Daily effective radiation in any time of the year falling upon the primary producer at latitude 35° on a fine day and cloudy day. A: direct rays of the sun, B: diffused light. A and B make a total daylight under the direct sunshine.

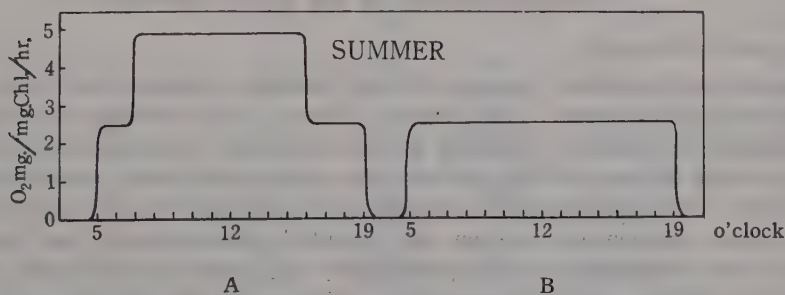


Fig. 7. Daily changes of the photosynthetic rate at Station 6 obtained from Figs. 2, 5 and 6. A, on a fine day; B, a cloudy day in summer.

of the photosynthetic rate at Station 6 obtained from Figs. 2, 5 and 6. Daily photosynthetic value may now be obtained by integrating hourly photosynthesis over the entire day.

Since the light condition on the river bed varies conspicuously with the stations, the daily production should be calculated at each station. The foregoing procedure, therefore, seems to be too complicate for the determination of the primary production of the mountain river. Hence the equation originated by Monsi and Oshima²⁾ was introduced in this study for the calculation of the production. As seen in Fig. 7, the amount of hourly photosynthesis under the direct sunshine was roughly the same through the day time and the similar results also found on the cloudy day. From these facts, the daily net production was calculated by the following equations;

$$\begin{array}{ll} \text{on a fine day} & P_{nf} = a_{s+a} \cdot T + a_a \cdot t - 24 \cdot r \\ \text{on a cloudy day} & P_{nc} = a_a \cdot t - 24 \cdot r \end{array}$$

where a_{s+a} and a_a being the hourly real photosynthesis per unit amount of chlorophyll under total daylight and diffused light, T and t being photosynthetic time under the direct sunshine and diffused light, r , the respiration. As mentioned previously, T and t were determined by means of the overlay. The intensity on the river bed under direct sunshine was estimated from Fig. 6. As described in the previous section, the diffused light intensity in the open was assessed as 10 klux and that of the river bed was able to be calculated by means of the ratio of the open sky area to the whole hemisphere on the photograph. Under the cloudy day, the light intensity was assumed as that of the diffused light of fine day. These assumptions on the light condition are too rough but reasonable ecologically. Since the hourly photosynthetic rate should be determined from the photosynthesis-light curves, the precise information on the pattern of photosynthesis-light curve is indispensable. Because of insufficiency in data on the curve, the calculation in the present study was carried out by using the curves in Fig. 5.

Assuming the cloudy and fine day occur alternately in a month, the monthly production in the unit area on the river bed can be obtained approximately as follows;

$$P_N = (P_{nf} + P_{nc}) \cdot C \cdot D / 2 - R \cdot C \cdot D$$

where C is the chlorophyll amount in the unit area on the river bed, D is days in month, P_{nf} and P_{nc} are daily gross production on a fine day and cloudy day.

Productivity and Dry Matter Production in the Mountain Section of the River Arakawa

1. Potential productivity.

Seasonal and locational changes in the potential photosynthesis were estimated by the foregoing procedure. As shown in Table 1, it is apparent that the potential photosynthesis is greater in spring and summer than in autumn and winter. This referred to the increase of the duration time under the direct sunshine and to the acceleration of the photosynthetic rate due to the increase of the water temperature. The foregoing relationship between the potential photosynthesis and light condition was also found locationally. The productivity was lower in the canyon section than in the lower section, and furthermore the values of the shady stations were smaller than those of the bright stations within the same river section. According to the present study, there was a rough parallel correlation between the productivity and the standing crop in the mountain section, thereby it can be surmised, that the light condition is the most important limiting factor for the production in the mountain river. However, it seems unreasonable to discuss the causes determining the standing crops through only the productivity, because the standing crop is determined by the interplay of the production rate and the destruction rate. As can be seen in Fig. 8, the parallel correlation is not so distinctly fine. The diversity in the standing crops of the canyon and lower section was more definite than that in the productivities. The foregoing discrepancy may suggest the existence of the other habitat factors affecting on the standing crop. As mentioned in a previous paper¹⁾, the physical actions such as scraping and rubbing have an important role on the reduction of the standing crop.

Table 1. Seasonal and locational changes in the potential photosynthesis in the mountain section of the River Arakawa.

Station	Sky light %	Photosynthetic activity, O ₂ mg./mg. chl./day (Gross production, glucose g./m. ² /day)				
		Feb.	May	Aug.	Nov.	Average
1	22	14.6 (1.63)	33.8 (0.61)	30.7 (1.55)	14.0 (1.14)	23.3 (1.23)
2	33	18.5 (0.26)	42.0 (0.40)	39.4 (0.44)	17.6 (0.78)	29.4 (0.47)
3	30	17.2 (0.00)	35.3 (0.56)	31.8 (0.36)	17.0 (0.33)	25.3 (0.31)
4	42	19.2 (0.24)	45.3 (0.55)	41.0 (0.46)	19.2 (1.33)	31.2 (0.65)
5	51	20.3 (0.28)	47.0 (0.13)	46.1 (0.09)	20.0 (0.68)	33.4 (0.30)
6	46	19.4 (1.31)	45.3 (0.25)	42.8 (0.28)	19.4 (1.96)	31.7 (0.95)
7	34	17.4 (0.13)	46.5 (0.44)	39.9 (0.34)	17.4 (0.23)	30.3 (0.29)
8	47	20.3 (0.09)	44.6 (0.29)	43.9 (0.74)	19.3 (1.19)	32.0 (0.58)
10	64	21.1 (0.03)	53.4 (1.35)	48.0 (2.52)	21.0 (0.30)	35.9 (1.05)
11	56	21.1 (0.26)	49.9 (1.12)	47.9 (1.48)	21.0 (1.70)	35.0 (1.14)
12	65	21.1 (0.25)	50.4 (0.47)	46.4 (0.57)	21.0 (0.33)	34.7 (0.41)
13	66	21.1 (0.32)	51.2 (0.14)	47.7 (2.19)	21.0 (0.43)	35.3 (0.77)
14	72	21.1 (0.00)	52.2 (0.83)	49.0 (3.03)	21.0 (2.07)	35.8 (1.48)
15	76	21.1 (0.40)	54.6 (0.56)	50.2 (1.46)	21.0 (2.72)	36.7 (1.29)
16	63	21.1 (0.30)	49.7 (0.70)	46.4 (2.00)	21.0 (2.11)	34.6 (1.28)
17	66	21.1 (0.20)	50.5 (1.33)	47.3 (6.21)	21.0 (2.15)	35.0 (2.47)
18	71	21.1 (0.11)	53.5 (0.30)	49.0 (5.05)	21.0 (3.35)	36.2 (2.20)
19	76	21.1 (0.14)	55.7 (1.67)	50.7 (6.32)	21.0 (5.57)	37.1 (3.43)
20	82	21.1 (0.08)	55.9 (0.47)	51.8 (1.51)	21.0 (0.32)	37.5 (0.60)

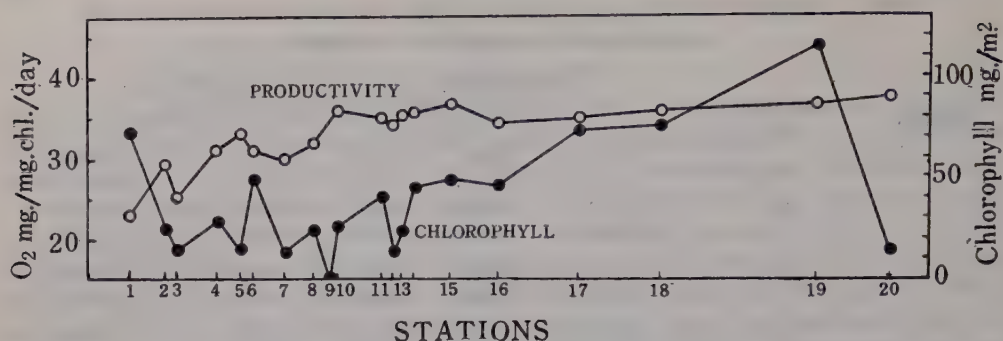


Fig. 8. Correlation between standing crop and productivity. Open circles: locational changes in annual average productivity. Filled circles: locational changes in average chlorophyll amount.

2. Dry matter production in the mountain section of the River Arakawa.

a. Gross primary production: As shown in Table 1 and Fig. 9, the gross primary production per square meter of the river bed was calculated from the chlorophyll amount and the potential productivity. The mean value of the gross production ranged from 0.3 to 1.2 g. glucose/m.²/day in the canyon section and 0.4 to 2.5 g. glucose/m.²/day in the lower section, except the abnormally large values of 3.4 g. glucose/m.²/day obtained at Station 19.

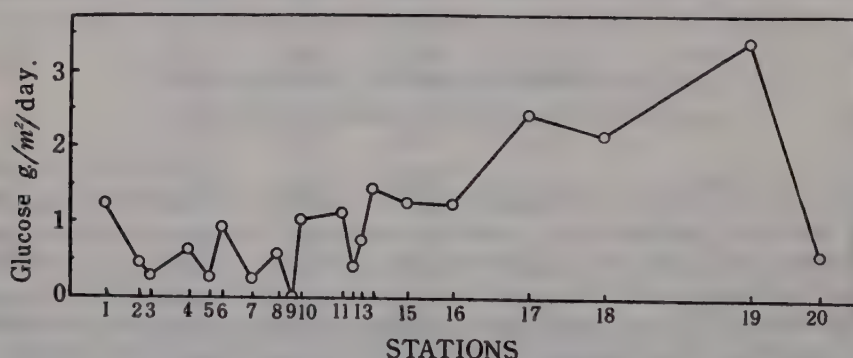


Fig. 9. Locational changes in average gross primary production per square meter of the river bed in the mountain section of the River Arakawa.

As the annual gross primary production, 0.21 kg. glucose/m.² (24,760 kg. glucose/119,000 m.², see Table 2) in the canyon section and 0.81 kg. glucose/m.² (1,883,790 kg. glucose/2,316,000 m.²) in the lower section were deduced, respectively. According to the data obtained by Hogetsu and Ichimura⁸), the annual gross production in eutrophic lake Suwa is 0.57 kg. glucose/m.². Manning and Juday⁹) reported 0.8 kg. glucose/m.²/year in the eutrophic lake Weber. In the mesotrophic lakes of Japan, the annual gross production has been measured 0.2 to 0.4 kg. glucose/m.² (Ichimura's unpublished data). Therefore, the productivity of the River Arakawa can be compared with those of the mesotrophic lakes within the canyon section and the eutrophic lakes within the lower section.

Total gross production in the whole area of the mountain section of the River Arakawa was calculated from the annual gross production at each station and the

Table 2. Dry matter production in the mountain section of the River Arakawa.

Division order	River bed area ha./order	Glucose ton/order/year (Glucose kg./m. ² /year)		
		P _G	R	P _N
1	50	2.25 (0.45)	1.95 (0.39)	0.30 (0.06)
2	150	2.55 (0.17)	1.80 (0.12)	0.75 (0.05)
3	90	0.99 (0.11)	0.81 (0.09)	0.18 (0.02)
4	175	4.20 (0.24)	2.63 (0.15)	1.57 (0.09)
5	140	1.54 (0.11)	1.12 (0.08)	0.42 (0.03)
6	210	7.35 (0.35)	5.25 (0.25)	2.10 (0.10)
7	200	2.20 (0.11)	1.20 (0.06)	1.00 (0.05)
8	175	3.68 (0.21)	2.28 (0.13)	1.40 (0.08)
Canyon section	1190	24.76 (0.21)	17.04 (0.14)	7.72 (0.07)
10	150	5.70 (0.38)	2.55 (0.17)	3.15 (0.21)
11	250	10.50 (0.42)	5.50 (0.22)	5.00 (0.20)
12	150	2.25 (0.15)	1.20 (0.08)	1.05 (0.07)
13	375	10.50 (0.28)	5.25 (0.14)	5.25 (0.14)
14	765	41.30 (0.54)	19.89 (0.26)	21.41 (0.28)
15	1200	56.40 (0.47)	32.40 (0.27)	24.00 (0.20)
16	1620	76.14 (0.47)	42.12 (0.26)	34.02 (0.21)
17	2550	229.50 (0.90)	112.20 (0.44)	117.30 (0.46)
18	5600	448.00 (0.80)	235.20 (0.42)	212.80 (0.38)
19	7500	937.50 (1.25)	487.50 (0.65)	450.00 (0.60)
20	3000	66.00 (0.22)	30.00 (0.10)	36.00 (0.12)
Lower section	23160	1883.79 (0.81)	973.81 (0.42)	909.98 (0.39)

area of the river bed. For calculation, the watercourse was divided into the regular orders and the area of the each order was estimated from the river course map. The productivity of the each order in the watercourse was represented by dry matter at the chlorophyll sampling station within the order. The calculation results are summarized in Table 2. The area of the river bed was 119,000 m.² in the canyon section and 2,316,000 m.² in the lower section. The annual gross production on these river beds was 25 ton glucose in the former section and 1884 ton glucose in the latter.

b. Net primary production: The amount of the respiration was computed from the data obtained in the dark bottle to be about 0.6–0.9 mg. O₂/mg. chl./hr., i.e. 1/5 of the photosynthetic rate at the light saturation point. By using the above values, the daily average respiration was calculated at 0.2 to 1.0 g. glucose/m.² in the canyon section and 0.3 to 2.0 g. glucose/m.² in the lower section. In the whole watercourse, the organic matter consumed through the respiration was 17 ton glucose in the canyon section and 974 ton glucose in the lower section (see Table 2).

Finally, the net production in the former section was 8 ton glucose and 910 ton glucose in the latter section.

Summary

Primary production in the mountain section of the River Arakawa was estimated from chlorophyll amount and light data obtained from the photographs of celestial hemisphere and the photosynthesis–light curves of the river algae.

1. Annual gross production was 0.21 kg. glucose/m.² in the canyon section and 0.81 kg. glucose/m.² in the lower section. These values can be compared with those in the ordinary mesotrophic lakes and eutrophic lakes in Japan. In the whole watercourse of the mountain river, total sum of the gross production was 25 ton glucose in the canyon section and 1,884 ton, glucose in the lower section, and the amount of the respiration was 17 ton and 974 ton, respectively. As a net-production, finally, 8 ton and 910 ton were obtained in the former and latter sections.

2. Among the habitat factors determining the productivity, the light condition on the river bed is most important and it is confirmed through the analysis of the locational changes in the potential photosynthesis of the sessile algae.

The author wishes to express his sincere thanks to Prof. H. Ito and Dr. S. Ichimura of Tokyo University of Education for their instructive advice and suggestion throughout the progress of this study.

References

- 1) Kobayasi, H., Bot. Mag. Tokyo 74: 228 (1961).
- 2) Monsi, M., and Oshima, Y., Jap. Jour. Bot. 15: 60 (1955).
- 3) —, and Saeki, T., *ibid.* 14: 22 (1953).
- 4) McConnel, J. W., and Sigler, W. F., Limnology and Oceanography 4: 335 (1959).
- 5) Hirayama, T., Theoretical Architecture (Japanese: Kenchiku-Sekkei Riron), Tokyo (1948).
- 6) Ichimura, S., Bot. Mag. Tokyo 71: 110 (1958).
- 7) Ryther, J. H., Limnology and Oceanography 1: 61 (1954).
- 8) Hogetsu, K., and Ichimura, S., Jap. Jour. Bot. 14: 280 (1954).
- 9) Manning, W. M., and Juday, R. E., Trans. Wis. Acad. Sci. Arts and Lit. 33: 363 (1941).

摘 要

小林 弘： 溪流の底生藻類群落の生産量について

前報で筆者は荒川の渓流域(勾配が急で河底が礫または小石で占められている部分)の底生藻類群落の現存量をクロロフィル量によって測定し、これと環境要因との関係を論じた。つづいて本報ではクロロフィル量を基にして生産力の算定を試みた。

底生藻類の生産にとっても光条件が最も重要な要因であるが、河川では特に付近の地形に支配されて、河底の光条件が場所ごとに異なっているため、その測定には特別な考慮がなされなければならなかった。これと荒川の底生藻類の光合成光曲線より算定した物質生産量は上流部で年間単位面積あたり $0.21 \text{ kg. glucose/m.}^2$ 、下流部で $0.81 \text{ kg. glucose/m.}^2$ であって前者は中栄養湖の値と、また後者は富栄養湖の値と一致している。これらの値から 0.14 kg. 、 0.42 kg. を呼吸としてそれぞれ差引くと、 0.07 kg. 、 0.39 kg. が純生産として残される。したがって河底面積 $119,000 \text{ m.}^2$ の上流部では総生産量は年間 25 ton 、 $2,316,000 \text{ m.}^2$ の下流部では $1,884 \text{ ton}$ 、そのうち 17 ton および 974 ton が呼吸によって消費され、純生産量は 7 ton および 910 ton であると推定された。

計算の結果得られた理想生産力と現存量は藻体の剝離、流失、他の生物による捕食などによる消耗がなければ比例した関係にあるべきもので、荒川で見られた部分的な両者の差異はこれらの阻害要因の存在を示すものと思われる。(東京教育大学理学部植物学教室)

Analytical Studies on the Development of Foliage of a Plant Community*

by Toshiro SAEKI**

Received February 20, 1961

The stratifying clip method after Monsi and Saeki¹⁾ enabled us to reveal the productive structure of plant communities, such as characteristic features of vertical foliage distribution. The foliage distribution in a plant community plays one of the most important roles in the dry matter production, and accordingly it is an essential element of the productive structure. A theoretical equation presented in a previous paper²⁾ interpreted the establishment of the typical shape of the vertical foliage distribution in a plant community. The assumptions adopted there, however, are not satisfactory in direct evidence, and the curves obtained from the theoretical equation were, on account of their static nature, insufficient to interpret in a dynamic aspect the foliage development of a plant community.

The present paper deals with the establishment of the vertical distribution of leaves especially in connexion with the growth of individual leaves within a plant community. Discussions will be given on varied types of leaf distribution, mainly based on a theoretical equation of a previous paper³⁾ concerning leaf growth.

Vertical foliage distribution as related to leaf and stem growth

In a previous paper³⁾ the author presented a mathematical equation which provides the size or dry weight of individual leaf at any time and at any insertion along the main stem of a plant, when plastochrone, daily increase in the total leaf amount of the whole plant, and leaf dry weight per unit leaf area are given beforehand. An attempt has also been made in that paper to calculate theoretically the time drift of the daily increase of leaf area index (LAI) in a stand. The calculated time drift is

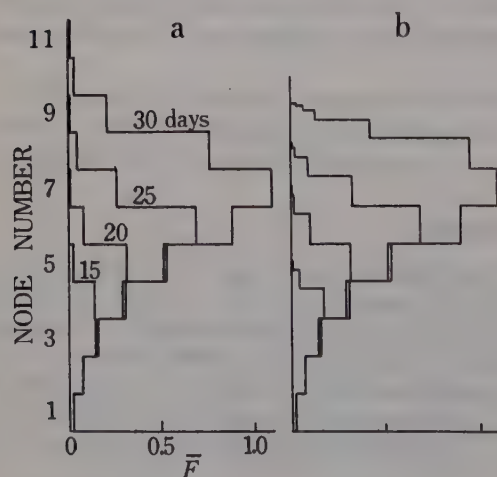


Fig. 1. (a) Theoretically calculated leaf amounts at different nodes at 15, 20, 25 and 30 days after the start of development of a plant community. Procedure of calculation is detailed in a previous report³⁾, where the same figure is shown in Fig. 9 but somewhat smoothed. Plastochrones are assumed 5 days from L_1 to L_4 , 4 days from L_4 to L_7 , 3 days from L_7 to L_{10} and 2 days from L_{10} to L_{13} . (b) The top parts of (a) are revised so that the ordinate can express the height of leaf insertion and a rectangle enclosed by node length and length in abscissa may be proportional to the leaf amount. Node length is reduced to $\times 0.8$, $\times 0.5$, $\times 0.25$ and $\times 0.125$, in order, from the 8th node to the younger ones.

* Supported by a Grant in Aid of Scientific Research of the Ministry of Education.

** Department of Botany, Faculty of Science, University of Tokyo, Hongo, Tokyo, Japan.

the same with Curve S of Fig. 4 in the present paper. When this daily increase of LAI is combined with plastochrone, the above-mentioned theoretical equation concerning leaf growth gives node number-leaf amount relations at different days as shown in Fig. 1-a. The figures resemble, in appearance, the vertical foliage distribution in a plant community, because the numerical order of the successive nodes in the ordinate runs roughly parallel to the height of each corresponding leaf, and because a plant community is composed of individual plants. It may not be expected, however, that all the internodes of a stem are the same in length, so that some revision must be made in order to obtain a real distribution shape of leaves.

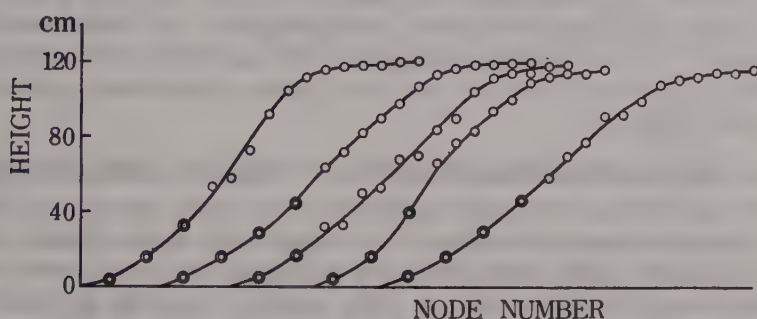


Fig. 2. The heights of successive nodes measured in five dominant individuals in a sunflower stand. A double circle denotes a node with opposite leaves; an open circle, a node with a leaf in alternate phyllotaxis. The first double circle of each curve stands for the first node.

Measurements were made of the heights of successive nodes in the plant individuals sampled from sunflower, green gram and reed stands. Out of them measurements in a sunflower stand are presented in Fig. 2, where both the top part and basal part of a stem have shorter internodes. The results in the other stands have showed similar trends. The shorter internodes at the top part, together with shorter petioles of young leaves, affect the shape of plant height-leaf size curve to become more compressed at the top part, while the shorter nodes in the basal part hardly change the shape of the distribution curve on account of smallness of the concomitant leaves. Thus, the transformation of the ordinate of node number in Fig. 1-a to the ordinate of node height provides the vertical foliage distribution as illustrated in Fig. 1-b. Dividing the values of abscissa by the number of the plants which are included in unit area will provide average values of leaf size or weight of all the constituent plants of a plant community. It is noteworthy that the figures in Fig. 1-b accord well with the real distribution patterns of foliage revealed by the stratifying clip method and that the shapes of the figures are invariable irrespective of growth stage.

Difference of foliage distribution between the herb- and grass-type communities

With regard to the vertical distribution of foliage in plant communities two major types have been distinguished¹⁾; the one, the herb type (Fig. 1-b), is the most familiar type, being found in a large number of plant communities. The other, the grass type, is met with many of grass communities. In the former the maximum foliage distribution lies at the upper part, while in the latter it lies at the lower part. As

intensively discussed in previous papers^{1,5)} grass plants allow much larger fraction of incident light to penetrate into the lower strata of the plant community than do herbaceous plants. Suppose the extinction coefficient of light penetrating in a grass-type community is e.g., 0.4 and that in a herb-type community is 0.8, at the lower part of the foliage the leaves exceeding 1.74 in cumulative LAI (leaf area integrated from the top of the plant community per unit land surface) should receive higher illumination in the grass-type community than in the herb-type community, while at the upper part the leaves up to that cumulative LAI should receive lower illumination in the former than in the latter. A grass community, therefore, will permit comparatively vigorous growth of suppressed small individuals or tillers, and play a part in enhancing foliage distribution at the lower strata, and consequently, in giving rise to a grass-type distribution. In this connexion reasonable is the assumption introduced in previous papers^{1,2)} that the vertical distribution of the foliage is established proportionally to the dry-matter productivity.

Density effect on the structural pattern of foliage in plant communities

Variant types of vertical foliage distribution have been reported in the experiments with different planting densities. In a stand with higher density the whole foliage is compressed at the upper part, while in a stand with lower density the foliage is distributed more uniformly over a wide range of vertical direction^{6,7)}. The phenomenon can be interpreted as follows: In the early growing stage foliage development is faster in a denser stand than in a sparser stand. In the former, therefore, severe light deficiency and resultant starvation cause a faster fall of the leaves in the lower strata, and consequently, the leaves can survive only at the upper part. In addition, a 'tendency towards equalization of plant height' in a dense population⁸⁾ may intensify the assembling of the foliage at the upper strata. Besides, the individuals under high planting density are lower in productivity on account of intense mutual shading, and it is expected to effect the lowering of the rate of leaf emergence as reported in a preceding paper³⁾. The retardation of leaf emergence will, when it occurs as observed in a tobacco plant under dense planting³⁾ (see Fig. 3), result in a flat

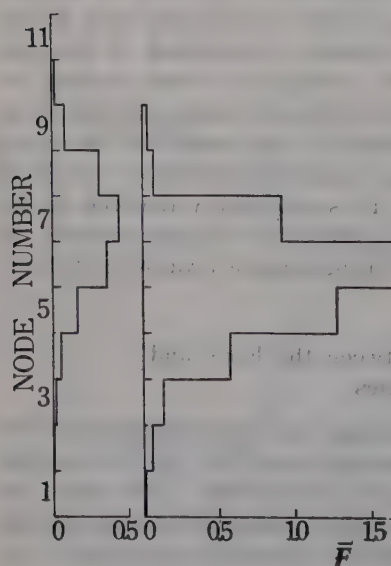


Fig. 3. Leaf amounts at successive nodes of stems in two stands different in density. The values were measured in tobacco plants 39 days after sowing. The left; a sparse stand of which mean available space per plant is 360 cm.² and the plants with such sufficient space are assumed to be able to grow in the same manner as a solitary plant. The right; a dense stand of which mean available space per plant is 36 cm.² (see Saeki³⁾).

shape of foliage distribution at the top part.

In order to find out the difference of vertical leaf distribution in two plant communities started from different planting densities (1:10), a calculation was attempted by using the theoretical equation concerning leaf growth presented in a previous paper³). Initial leaf area index was assumed to be 0.04 in a sparse stand (S) and 0.4 in a dense stand (D). The time drift of the daily increment of the total leaf weight (or area) was calculated in the same way as done in the above paper, and the results are shown in Fig. 4. On the 20th day the increment of LAI (ΔF) in stand D attains to the maximum. Provided that the leaves below compensation point are assumed to die thereafter, daily increase of new leaves remains constant but net increase of leaf mass is zero. The foliage distribution after 30 days in stand D is

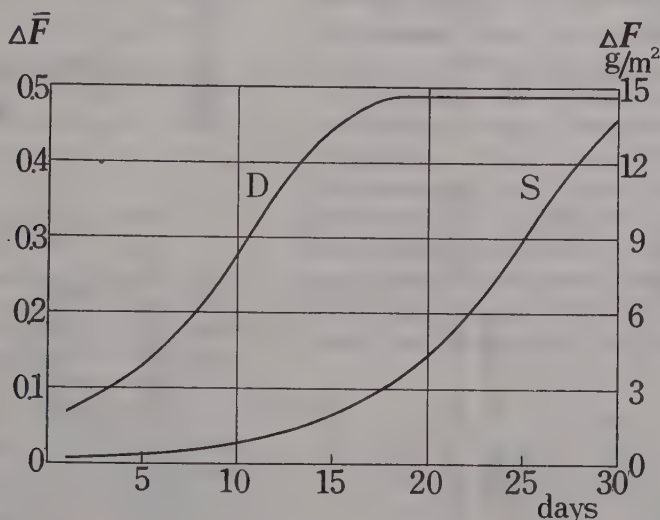


Fig. 4. Theoretically calculated daily increase of leaf area index or leaf dry weight in a sparse stand (S) and in a dense stand (D). The initial leaf area indices are 0.04 in the former and 0.4 in the latter.

illustrated in Fig. 5-D₁. There the hatched part corresponds to the leaves situated below mean daily compensation point. The ordinate expressing node number is subsequently revised into the ordinate of height of leaf insertion (Fig. 5-D₂). Theoretically the leaves below the chain line cannot produce dry matter positively, because the light intensities prevailing on these leaves are below compensation point, and the leaves will sooner or later perish to fall. In natural communities, however, the lowest surviving leaves of the constituent plants are different in height one another. Fig. 6 gives some examples for real plant communities. The lowest surviving leaf in each plant is distributed in the range of $\pm 30\%$ of the mean height of the lowest surviving leaves in a sunflower stand, $\pm 33\%$ in a reed stand, and $\pm 71\%$ in a young stand of green gram. When we apply $\pm 30\%$ to the above dense stand (D), some leaves succeed but the other fail to survive in the range between the two dotted lines in Fig. 5-D₂. Here are included 77% of the 4th stratum numbered from the base, 100% of the 5th stratum and 99% of the 6th stratum. Provided that the frequency curve as shown in Fig. 6 is approximated by a straight line, it follows that within the above range the 4th stratum loses 86.1% of its leaves; the 5th stratum, 54.0%;

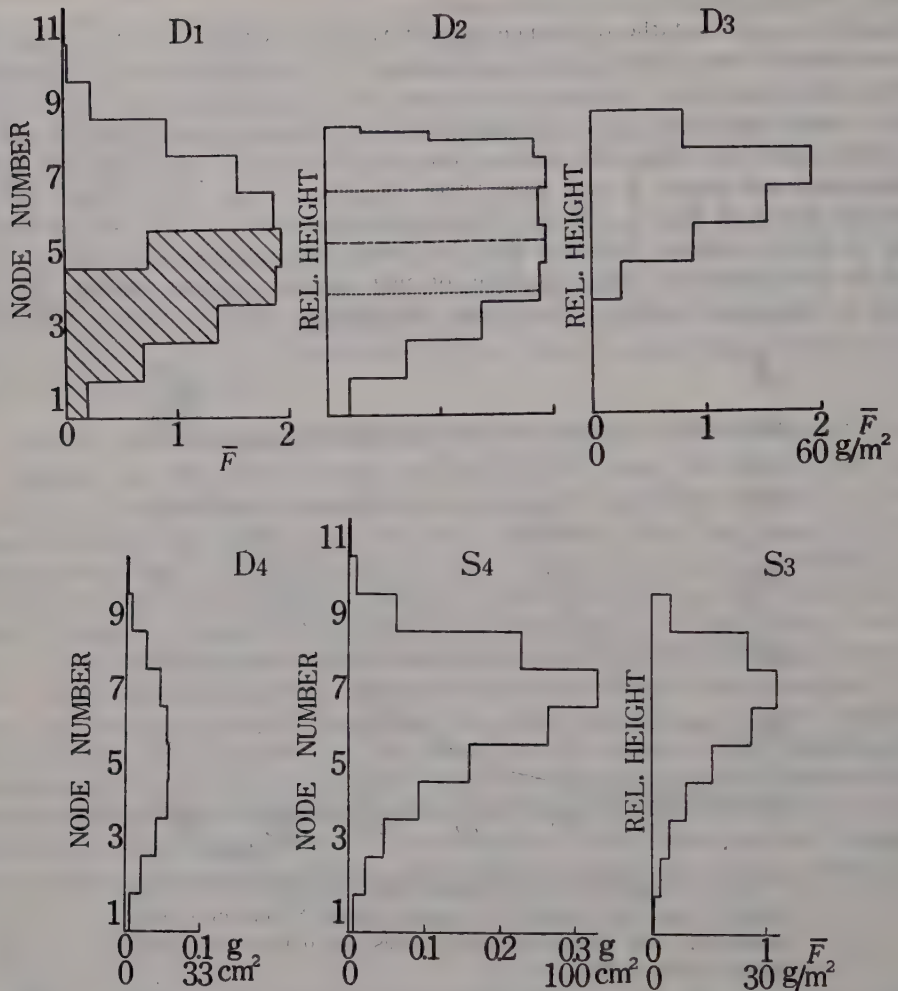


Fig. 5. Comparison of vertical leaf distribution between a dense stand (D) and a sparse one (S) 30 days after the start of development. D₁: Leaf amounts in LAI as related to node number in stand D. The hatched part is below compensation point. D₂: The ordinate is revised to express a relative height. The mean value of daily compensation point is at the level of the chain line, but the lowest surviving leaves practically range between the two dotted lines. D₃: The top part is divided into the same thickness of strata as the lower strata, so as to be comparable with the foliage distribution revealed by stratifying clip method. The lower part is also revised according to the frequency of heights of the lowest surviving leaves (see Fig. 6). D₄: Leaf sizes or dry weights at different nodes of stem in an averaged plant in stand D. S₃: Vertical distribution of leaves in stand S as related to relative height. Comparable with D₃. S₄: Leaf sizes or dry weights at different nodes of stem in an averaged plant in stand S. Comparable with D₄.

the 6th stratum, 17.8%. Naturally, 100% leaves are lost below the lower dotted line. Taking these values into the calculation, we finally obtain Fig. 5-D₃ in a comparable form with the result obtained by the stratifying clip method. The foliage distribution of the sparse stand (S) is illustrated in S₃ (in the same scale with D₃), where in

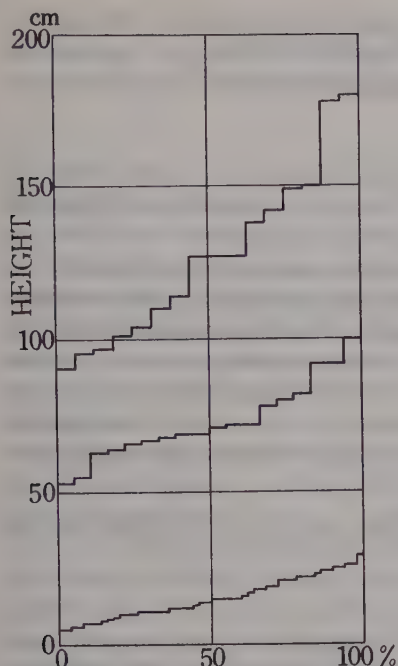


Fig. 6. The height of the lowest leaf remaining alive in each plant, of 16 samples in a reed stand ca. 2.6 m. high (A), of 18 samples in a sunflower stand ca. 1.2 m. high (B) and of 50 samples in a green gram stand ca. 0.6 m. high (C). The samples are arranged on the abscissa in the order of height.

Similarity of vertical needle distribution in coniferous trees

Pinus densiflora, *Chamaecyparis obtusa* and *Picea jezoensis* have been reported to exhibit the herb type in the vertical distribution of needles^{10,11,12}). Coniferous trees in general bear long-lived needles, whose expansion takes place almost simultaneously at all branches. New needles are distributed in different proportions in main branches. It was recognized from the measurement in each of the main branches that the vertical weight distribution of the current-year-needle roughly followed a symmetric pattern (Fig. 7), as did the daily weight (or areal) increment of an individual leaf in herbs. This appears also the case in the data reported by Shidei *et al.*¹⁰). The reason why such pattern is established in forest trees is undoubtedly

this stage of growth all leaves enjoy illuminations more than daily compensation point. The comparison of D_3 with S_3 indicates that the density effect upon the vertical foliage distribution has been successfully analysed. D_4 and S_4 in Fig. 5 represent the size of each individual leaf in an averaged plant in stands D and S, respectively. It is natural from the figure that as experienced in field works the individuals in the dense stand should have smaller leaves than have the individuals in the sparse stand, in so far as different planting densities cause no difference in the time trend of leaf-emergence rate. In the same way, a plant in a weak light intensity, as under forest canopy, should bear smaller leaves on account of low productivity, so far as leaf-emergence rate is not much retarded and/or 'leaf dry matter index'⁹) is not so small as seen in many sun plants. Instead, if the leaf appearance is extremely delayed and/or the leaves become very thin seen in typical shade plants, leaf area should be rather larger.

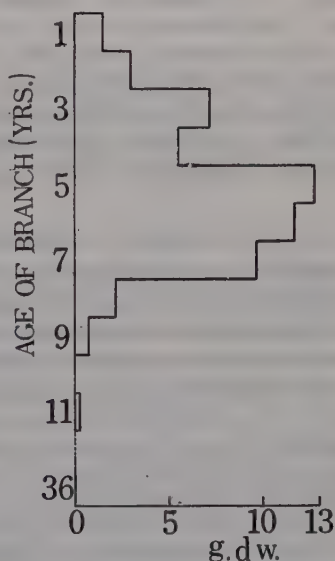


Fig. 7. Distribution of current-year-needles in an *Abies Mariesii* tree, measured at Mt. Shimagare, Nagano Prefecture. Numbers in ordinate mean the age of the main branches attached to the bole, and in inverse order, it approximately means the relative height at which the needles are held.

different from that in herbaceous plants, but the superficial coincidence of the pattern between herbs and coniferous trees will provide an apparent coincidence between the vertical leaf distribution in herbs and the vertical needle distribution in forest trees.

Summary

The development of foliage distribution in vertical direction in a plant community was interpreted by the equation concerning leaf growth presented in a previous paper³), in conjunction with stem growth. An analytical elucidation was given to varied types of foliage distribution met with natural plant communities or formed experimentally with different planting densities, on the basis mainly of 1) the relationship between light penetration and production of matter, 2) increasing plastochrone in the shade and 3) fluctuation in height of the lowest leaves surviving inside a plant community. The theoretical calculation of leaf growth indicated that leaf sizes of densely grown plants are, on account of the low productivity of an individual plants, smaller than those in sparsely grown plants, so far as leaf-emergence rate is the same in both the cases.

The author wishes to express his sincere gratitude to Prof. M. Monsi for his invaluable advice and encouragement.

References

- 1) Monsi, M., and Saeki, T., Jap. J. Bot. **14**: 22 (1953).
- 2) Saeki, T., and Kuroiwa, S., Bot. Mag. Tokyo **72**: 27 (1959).
- 3) Saeki, T., *ibid.* **74**: 70 (1961).
- 4) Kuroiwa, S., *ibid.* **73**: 865 (1960).
- 5) Saeki, T., *ibid.* **73**: 55 (1960).
- 6) Kuroiwa, S., and Monsi, M., J. Agr. Meteorol. **12**: 41 (1956).
- 7) Hogetsu, K., Oshima, Y., Midorikawa, B., Tezuka, Y., Sakamoto, M., Mototani, I., and Kimura, M., Jap. J. Bot. **17**: 278 (1960).
- 8) Hozumi, K., Koyama, H., and Kira, T., J. Inst. Polytech., Osaka City Univ., Ser. D. **6**: 121 (1955).
- 9) Totsuka, T., and Monsi, M., Bot. Mag. Tokyo **73**: 14 (1960).
- 10) Satoo, T., Nakamura, K., and Senda, M., Bull. Tokyo Univ. For. No. **48**: 65 (1955).
- 11) Satoo, T., and Senda, M., Bull. Tokyo Univ. For. No. **54**: 72 (1938).
- 12) Shidei, T., Studies on the productivity of the forest. I., Kokusaku Pulp-Ind. Co. Ltd., Tokyo, 65 pp. (1960).

摘 要

佐伯敏郎：植物群落における葉層の発達についての解析的研究

植物群落に層別刈取法を適用すれば、葉の量の垂直分布が常に特徴的な形をしているのがみられる。以前の報告²⁾ではこの形を二、三の仮定から理論的に説明した。しかしその仮定の証明は十分でなく、また葉の垂直分布の時間的発展の過程を十分にあらわしえなかった。そこで本論文では、一層具体的に前報³⁾の葉の生長式を用いてこの形の成立の説明を試みた。

前述の葉の生長式によれば、一本の植物または群落全体の総葉量の毎日の増加（例えば理論的に計算された第4図）と出葉速度がわかれば、各ふしにつく葉の生長量がわかる。各ふしの番号を縦軸にとり、横軸に葉量をとれば、縦軸はほぼ葉の高さを表わすので、葉の垂直分布の形にたものがえられる（第1-a図）。ただ葉の頂部の節間はつまっている（第2図）その補正を加えれば縦軸は真に相対的な高さを示すものとなり、層別刈取法によるものとよく一致したものをうる（第1-b図）。

下部のふくれたイネ科型の葉の垂直分布の成立の原因は、広葉型にくらべ群落下部への光の侵透がよく、そのため分けつや劣勢個体の生長が比較的よいためと説明した。密植すると、疎植した場合にくらべて葉が上部へおしつけられたようにかたまるが、この型の成立を補償点以下の葉の枯れ上がり、および生き残る葉の高さが一様でないこと（第6図）を入れて理論的につくりあげた（第5図 S_1, S_2, S_3 ）。木でも草と同じような葉層の分布型が多数報告されているが、シラビソでの測定結果では各主枝の一年間の針葉のふえが、見かけ上、草本の各ふしの一日の葉のふえ方と同じ形である（第7図）ことからくるものである。このことはただし群落状態でのみあてはまり、原因は草本と別のものである。草本で生産力が低下しても出葉速度がおそくならず、葉がうすくならぬような種類では、高密度の場合や、光の弱い場合、個体の生産力が小さいから、個々の葉も小になり（第5図 S_4 と D_4 の比較）逆に出葉速度が非常におそくなり、葉がうすくなる種類では、個々の葉が大きくなることが理論的に推論される。（東京大学理学部植物学教室）

Ecological Studies of *Sasa* Communities

III. Photosynthesis and Respiration of *Sasa kurilensis**

by Yasuyuki OSHIMA**

Received March 27, 1961

Photosynthesis and respiration are the most important functions which can decide the dry matter production of a plant or a plant community. The dry matter production is changeable with the changes of these functions which have intimate relations not only to the environmental factors, but also to the productive structure of the plant or the plant community. Moreover, the structure develops as the resultant of the functions.

On the basis of the dry matter production, the author has already clarified in a closed community of *Sasa kurilensis* its characteristic structural features and their changes with time^{1,2}). Further step of the investigation must be followed by the clarification of physiological functions: the seasonal changes of photosynthetic and respiratory activities as well as the changes with aging in relation to environmental factors, especially light and temperature. Concerning these problems, many studies have already been carried out in herbs and trees³⁻¹⁶), but no information is available in *Sasa*. Here the author wants to present many data obtained during his study of the physiology and ecology of the *Sasa* community.

Method

Photosynthesis and respiration were measured with a modified Boysen Jensen apparatus¹⁶). Measurements of the former were carried out in summer of 1960 and of the latter in various seasons of 1958-1960, in a closed *Sasa kurilensis* community which developed under the optimal distribution condition at Mt. Waisuhorun in the southern part of Hokkaido¹).

For the measurement of photosynthesis, one-year-old or newly formed leaves which situated at the upper layer of the canopy were cut at the base of petiole in water to secure the water absorption. The measuring conditions were saturated light intensity (30 kilolux) or sometimes varying illuminations, constant temperature (20°), and 0.03 vol. % CO₂ (see also Saeki⁹). The test leaves had to be renewed at intervals of about two hours because of the extreme susceptibility in photosynthetic activity of the detached leaves against the water deficiency.

Respiration was measured in the CO₂-free air in various organs: leaves, branches and main culms of various ages and whole rhizomes and roots. About 150-400 g. fresh weight of each organ in fresh weight were put in a dark chamber under moist condition after covering the cut surface with water-free lanolin. Air temperature of the chamber was kept at the same temperature of the outdoors. Temperature coefficient of respiration was determined in each organ. Samples taken under snow cover were brought to the laboratory packed with snow and kept in it till the measurement was made.

* Part of this investigation was supported by a Grant-in-Aid from the Scientific Research of the Ministry of Education.

** Department of Biology, Faculty of Science, Tokyo Metropolitan University, Setagaya, Tokyo, Japan.

When the room temperature is too low for the effective CO_2 absorption by 1/20 N KOH in the absorption vessel, the latter was heated up.

Photosynthesis

Gross and net photosynthetic activities of one-year-old and newly formed leaves measured in June-August are summarized in Table 1. In the same season light-photosynthesis curves were also determined (Fig. 1). New leaves began to foliate in

Table 1. Net and gross photosynthesis (at light saturation) and respiration of *Sasa kurilensis* leaves of various ages. Measured at 20°, in 1960.

Date	June 28	July 9	Aug. 18
Leaf age	1	1 cur.*	1** cur.***
Net photosynthesis (mg. CO_2 /50 cm. ² /hr.)	8.4	8.0 3.8	4.9 7.7
Respiration (mg. CO_2 /50 cm. ² /hr.)	1.1	1.0 2.4	0.6 1.5
Gross photosynthesis (mg. CO_2 /50 cm. ² /hr.)	9.5	9.0 6.2	5.5 9.2
Compensation point (kilolux)	1.5	— 3.7	— —

* young leaf; ** shade leaf; *** mature leaf.

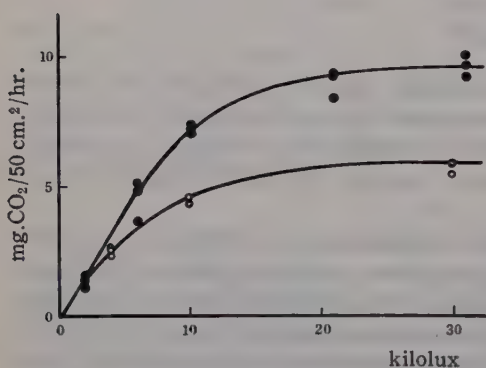


Fig. 1. Light intensity—gross photosynthesis curve in leaves of *Sasa kurilensis* at 20° and 0.3% CO_2 . Upper curve, one-year-old leaf; lower curve, young new leaf (see Table 1).

late June or early July, and their gross photosynthetic activity reached a maximum (ca. 9 mg. CO_2 /50 cm.²/hr.). From June to July, one-year-old leaves of upper layer of the foliage also showed the maximum photosynthetic activity. In evergreen broad-leaf trees and conifers, several workers^{7,8,10,13)} have already reported that the active leaves of these trees could maintain their maximum photosynthetic activities till the beginning of autumn. After Pisek and Winkler⁷⁾, subalpine conifers persisted in their maximum photosynthetic activity until the mean daily temperature fell to 0°, and then in winter, in very low level of photosynthetic activity. The activity recovered with air-temperature rising in the

spring. The mean annual air temperature for *S. kurilensis* community in this station is 4.4°, and the monthly mean is above 0° except for the snowfall season. Therefore, it may be probable that upper leaves of *S. kurilensis* persist in a considerably high, maximal photosynthetic activity during the vegetation period from the end of May to the beginning of November.

Maximum net photosynthesis observed in the active leaves of *S. kurilensis* was about 8 mg. CO_2 /50 cm.²/hr. under the conditions of light saturation and 20°. This falls in the range of the mean values in herbaceous plant of 7-8 mg. CO_2 , and is higher

than those in trees of 5–6 mg. CO₂ in the same unit, though those values were obtained at 25°¹⁷).

As shown in Fig. 2, the dry weight per 100 cm.² leaf area of newly formed leaves increased from 0.45 g. to 0.80 g. in the period from June 27 to October 15, and decreased slightly thereafter and during the snowfall season. With thawing at the end of May the dry weight of the one-year-old leaves rapidly increased, and reached a maximum value of 1.03 g./100 cm.² (July 8). So the photosynthetic rate per unit dry weight decreased with aging. New development of new leaves above one-year-old leaves brought about the decrease of dry weight as well as of photosynthetic activity to the latter (Table 1 and Fig. 2).

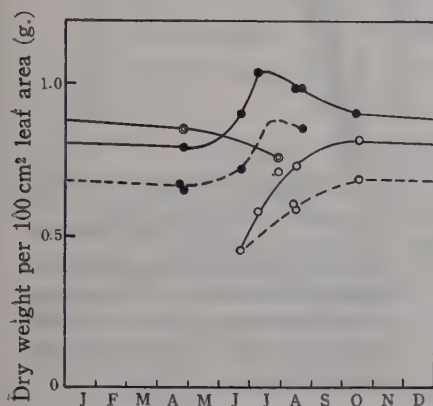


Fig. 2. Seasonal changes of dry weight per 100 cm.² leaf area of sun and shade leaves in *S. kurilensis*. Solid lines, sun leaves; broken lines, shade leaves. Open circles, new leaves; solid circles, one-year-old leaves; double circles, two-year-old leaves.

the similar condition, except for air movement of 0.020 m./sec. Lacking in detailed temperature data, however, presents the correction of the photosynthetic rate obtained.

New leaves of *Sasa nipponica*, *nikkoensis* and *oseana* are thinner than those of *S. kurilensis* in summer (Table 2 and see also a previous paper¹), and this may suggest that the photosynthetic activity on an area basis in those *Sasa* species is lower than that in *S. kurilensis*.

As mentioned before, the hourly photosynthetic activity of *S. kurilensis* leaves persisted at a certain level during the vegetation period. The daily rate of photosyn-

The seasonal changes in dry weight of shade thin leaves were about the same as those of sun leaves (Fig. 2). Younger leaves showed higher rate of respiration, higher compensation point and lower photosynthetic activity; with maturing of the leaves photosynthetic activity increased and respiratory rate and compensation point decreased (Table 1), and the light-photosynthesis curve showed characteristics of sun leaves, as already seen in other plant species^{6,8,9,14,15}).

Kuroiwa¹⁴) reported that the leaf temperature determined thermoelectrically in the assimilation chamber immersed in running water under a condition of 30 kilolux illumination and 0.006 m./sec. air movement was in *Fagopyrum esculentum* 2.7°, and in *Abies* 2.0° higher than the air temperature in the chamber of 20°. In consideration of these data, it may assumed that a similar slight temperature elevation occurred in the *Sasa* leaf whose assimilation was measured under

Table 2. The mean dry weight per unit leaf area of new leaves in four species of *Sasa*.

Species	Date	g. Dry weight/100 cm. ²
<i>S. nipponica</i>	Aug. 27	0.48
<i>S. nikkoensis</i>	Aug. 13	0.50
<i>S. oseana</i>	Aug. 10	0.62
<i>S. kurilensis</i>	Aug. 15	0.73

thesis, however, should change seasonally with the changes in day length and in the amount of daily solar radiation. The daily gross photosynthesis—relative light intensity curve for each month of the vegetation period was constructed by Saeki's method¹⁷⁾ for the hourly photosynthesis (the upper curve in Fig. 1) and the daily solar radiation at the station. The latter was estimated from the mean daily march of illumination observed by Hirayama¹⁸⁾ in Tokyo in consideration of the height of sun at 43°N and the transmissibility of air. The mean daily photosynthesis, which is represented as gain in $(C_6H_{10}O_5)_n$ for a direct comparison with plant growth in dry weight, can attain in 100% light a maximum of 15.8 g./m.² in May, June and July, and a minimum of 11.8 g./m.² in November.

In these calculations, however, the temperature factor which modifies seasonally photosynthesis in the field was not considered. Monthly mean temperatures in May, October and November were respectively 8.6, 7.4 and 0.2° at the station, being far less than 20° for the photosynthesis measurement. Although the daytime temperature at which the plant assimilates must generally higher than the said temperatures, the mean daily photosynthesis curves for these months are to be fairly lower than those illustrated in Fig. 3. Concerning this the winter will discuss in the next paper.

Respiration

The seasonal changes in respiration rate of leaves and main culms of various ages and of whole rhizomes and roots per g. dry weight per hr. at 20° were illustrated in Figs. 4 and 5. Values of respiration of branches of each age and seasonal changes in them are very much similar to those of culms.

The maximum respiration rate of new culms was observed at the end of May when the shoots were about 1-2 cm. high, and that of new leaves, at the end of June immediately before the unfolding. They were of the same value amounting to 6.5 mg. CO₂/g.d.w./hr. (see Fig. 5b). Respiration rate of these new organs decreased with time till November, as seen in the various organs in other plants^{4,12,15}).

After thawing, the respiration rate of the organs of one-year-old or more, and of whole organs began to increase and reached a maximum when the reserved substance was violently transformed into newly formed organs. The maximum value of one-year-old and many-year-old main culms, branches and leaves was seen at the end of June, while that of whole rhizomes and roots at the beginning of July. After the respiration rate decreased gradually with time till November, and a nearly constant low value of respiration rate was maintained during the season of snow accumulation (see Figs. 4 and 5). Ratios of maximum value to winter value in respiration of leaf, culm, branch, rhizome and root were 1.3, 2.0, 2.0, 1.5 and 2.7, respectively.

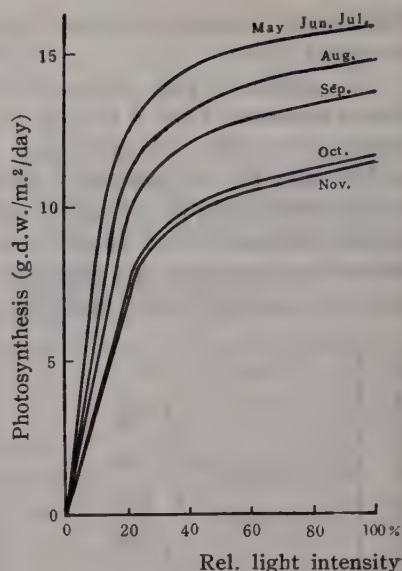


Fig. 3. Relative light—daily gross photosynthesis curves of active leaf of *Sasa kurilensis* under the light conditions at Mt. Waisuhorun, southern Hokkaido.

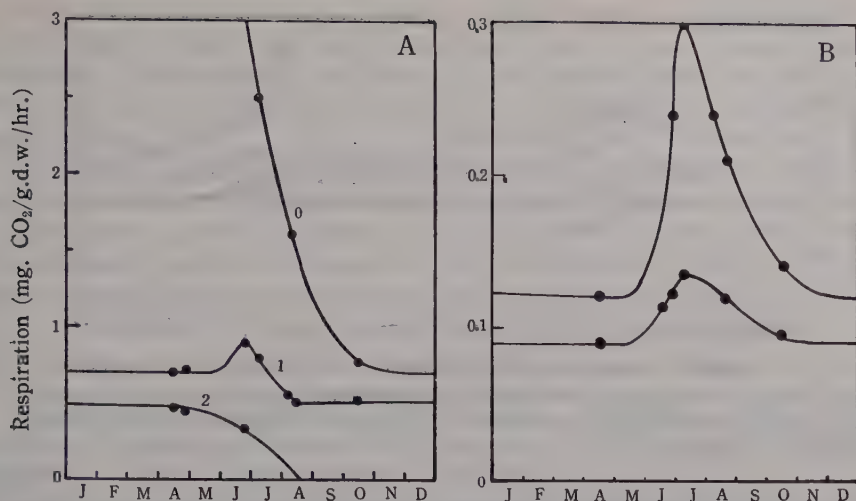


Fig. 4A; Seasonal change of respiration rate at 20° of *S. kurilensis* leaves of each age. Numerals at the curves indicate age of leaves.

B; Seasonal change of respiration rate at 20° of the rhizomes and roots. Upper curve, roots; lower curve, rhizomes.

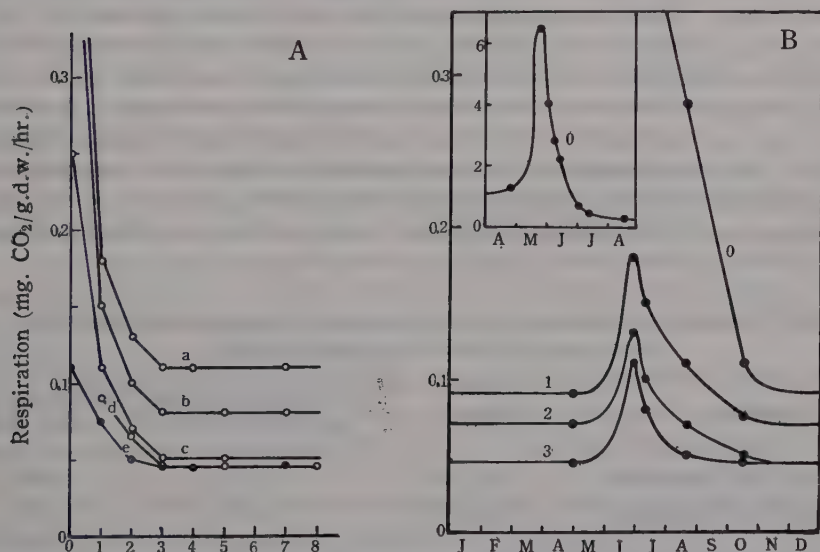


Fig. 5. A; Relation between the respiration rate at 20° and the age of main culms of *Sasa kurilensis*. a, June 27; b, July 7; c, Aug. 19; d, Oct. 15; e, April 29.

B; Seasonal change of respiration rate at 20° of the main culms of each age. Numerals at the curves indicate culm age.

Aging brings about the decrease of respiration rate in leaves, culms and branches. After three years, however, culms and branches keep almost constant respiration rates (see Figs. 4a and 5b). It is generally known that the thickening growth decreases the respiration rate of branch and trunk^{4,14}, probably for the sake of increase in the volume of non-living tissue. In the culm of *Sasa* observed, however,

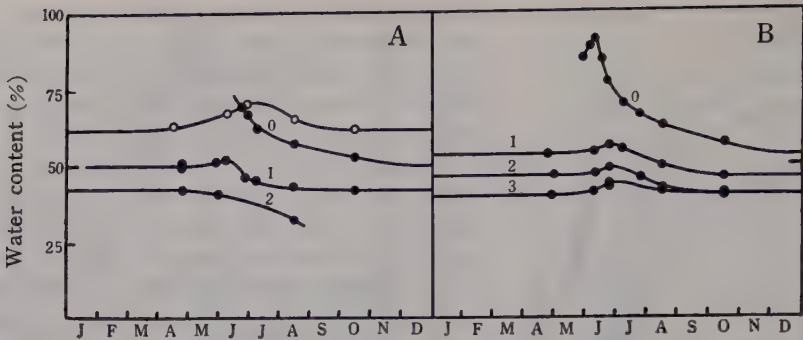


Fig. 6. Seasonal change of water content of each organ of *Sasa kurilensis*. (A), leaves of each age (solid circles) and rhizomes (open circles); (B), main culms of each year of age (or branches of each age). Numerals at the curves indicate of culm age.

there occurred neither thickening growth nor elongation after two years, while the dry weight increased during first three years. Furthermore, as seen in Fig. 6, the water content of each organ, except for the newly formed one, also showed the maximum value at the time when rapid transformation occurred, and main culms and branches more than three years old maintained unchangeable water content regardless of aging. These trends well agree with the results obtained in the respiration rate (Figs. 4 and 5b). From the above facts, it is possible to say that after three years the quantitative ratio of living tissue to non-living tissue in each organ is almost constant and the living tissue keeps its activities unchanged. This may be the main reason for the characteristic time trends of the respiration rate observed in *S. kurilensis*. The maintenance of a certain respiration rate of old non-photosynthetic systems, together with the large amount of the systems, causes a very high annual respiration loss to the whole community, which will be discussed in the next paper.

Table 3. Comparison between *Sasa kurilensis* and *S. nipponica* in respiration of one-year-old leaves and of new culms.

Species	Date	Respiration rate in mg. one-year-old leaves	CO ₂ /g.d.w./hr. New culms
<i>S. kurilensis</i> (Mt. Waisuhorun)	Aug. 15	0.50	0.28
<i>S. nipponica</i> (Mt. Kirigamine)	Aug. 21	0.52	0.27

Table 4. Temperature coefficients of respiration in various ranges of temperature.

Organ	Age	Date	Range of temp.	Q ₁₀	Organ	Age	Date	Range of temp.	Q ₁₀
Leaf	0 year	Oct. 14	11.0—21.2°	2.2	Culm	0 year	Oct. 15	13.1—23.2°	1.9
Leaf	0	Oct. 14	17.7—27.1	2.0	Culm	1	Jun. 26	10.0—19.1	2.0
Leaf	1	Apr. 28	11.2—20.0	2.1	Culm	3	Apr. 27	3.0—10.0	2.6
Leaf	1	Apr. 28	3.0—10.0	2.6	Culm	3	Apr. 27	11.1—20.0	2.0
Leaf	1	Apr. 28	3.0—20.0	2.3	Culm	3	Apr. 27	3.0—20.4	2.2
Leaf	1	Jun. 26	10.0—20.0	2.3	Rhizome Whole		Apr. 27	10.8—19.5	2.0

Respiration rate measured in winter at 20° of leaves, non-photosynthetic system of aerial part, rhizomes and roots were 0.62, 0.066, 0.09 and 0.11mg. CO₂/g.d.w./hr., respectively. The summer respiration of one-year-old leaves and new culms were almost the same in respective organs in *Sasa kurilensis* and *S. nipponica* (Mt. Kirigamine) (Table 3).

Temperature coefficient of the respiration rate was determined in every season in each organ of various ages. Some of the data obtained were summarized in Table 4. There was no great difference as to Q₁₀ obtained in other plants, regardless of wide ranges of ages and seasons; at 10-20°, 1.9-2.2, and at 3-10°, ca. 2.6. Leaves showed slightly higher Q₁₀ than that of non-photosynthetic organs.

Summary

Physiological characteristics, photosynthesis, respiration, water content, and thickness of leaves were measured in *Sasa kurilensis* of a closed community at Mt. Waisuhoron, 60 km. W from Sapporo, Hokkaido.

1. Gross photosynthesis of new leaves unfolded late in June increased and reached a maximum value of 9.0-9.5 mg.CO₂/50 cm²./hr. at the middle of August. This high value persisted till the next July, except for the snow season from the beginning of November to the end of May. With the development of the new leaves the photosynthetic activity of one-year-old leaves decreased in August.

2. The new leaves showed higher respiration and higher compensation point, and rather lower photosynthetic activity, than the matured leaves.

3. After foliation the thickness of leaves in the upper canopy increased till July of the next year except for the period under snow accumulation, and thereafter it decreased slightly till the shedding in the following summer.

4. Relative light intensity—daily gross photosynthesis curves for active leave have been constructed on the basis of the hourly photosynthesis curve and the daily march of illumination at the station in each month of vegetation period.

5. Respiration rate and water content in each organ increased rapidly with thawing, and reached their maximum values late in June or early in July. Afterwards they decreased gradually till the snow period when they maintained nearly constant low levels under snow accumulation.

6. The respiration rate and water content of young leaves and culms decreased with aging. After three years, however, a constant respiration rate was maintained.

7. Q₁₀-values in each organ were 1.9-2.2 at 10-20°, and ca. 2.6 at 3-10°.

The author should like to express his sincere thanks to Prof. K. Hogetsu of Tokyo Metropolitan University and Prof. Monsi of the University of Tokyo for their valuable advice and suggestion. His thanks are also due to Mr. I. Matsuoka from whom he has received many helps during the work in Hokkaido.

References

- 1) Oshima, Y., Bot. Mag. Tokyo **74**: 199 (1961).
- 2) —, ibid. **74**: 280(1961).
- 3) Willstätter, R., und Stoll, A., Untersuchungen über die Assimilation der Kohlensäure, Berlin (1918).
- 4) Möller, C. M., Müller, D., and Nielsen, J., Det forstl. Forsøgsvaesen **21**: 253, 273, 327 (1954).
- 5) Kasumoto, T., and Sakimoto, M., Bull. Educ. Rec. Inst., Kagoshima Univ. **6**: 139 (1954).
- 6) —, Jap. J. Ecol. **7**: 126 (1957).
- 7) Pisek, T., and Winkler, E., Planta **51**: 518 (1958).
- 8) Saeki, T., and Nomoto, N., Bot. Mag. Tokyo **71**: 235 (1958).
- 9) —, ibid. **72**: 404 (1959).
- 10)

Nomoto, N., Kasanaga, H., and Monsi, M., *ibid.* **72**: 450 (1959). 11) Tazaki, T., *ibid.* **72**: 68 (1959). 22) Midorikawa, B., *Ecol. Rev.*, **15**: 83 (1959). 13) Bourdeau P. F., *Ecology* **40**: 63 (1959). 14) Kuroiwa, S., *Bot. Mag. Tokyo* **73**: 152 (1960). 15) Hogetsu, K., Oshima, Y., Midorikawa, B., Tezuka, Y., Sakamoto, M., Mototani, I., and Kimura, M., *Jap. J. Bot.* **17**: 278 (1960). 16) Boysen Jensen, P., *Die Stoffproduktion der Pflanzen*, Jena (1932). 17) Saeki, T., *Bot. Mag. Tokyo* **73**: 55 (1960). 18) Hirayama, T., *Theoretical Architecture (Japanese)*, Tokyo (1948).

摘 要

大島康行: ササ群落の生態学的研究 III. チシマザサの光合成と呼吸

すでに報告した^{1,2)} 北海道ワイスホルン山のおなじチシマザサの純群落地で、チシマザサの葉の光合成能力、葉の厚さ、および各器官の各年令別の呼吸能力、含水量を測定した。

葉の光合成能力は6月下旬新葉の展開後、しだいに増大し、真の光合成能力は8月に飽和光、 20° で $9.0 \sim 9.5 \text{ mg. CO}_2/50 \text{ cm.}^2/\text{hr.}$ の最高値に達する。この最高値は冬期ササが雪の中に埋もれている期間をのぞいて、翌年の7月まで続き、つぎの新葉が群落の葉冠の上に展開しおわる8月以後低下する。若い葉は壮葉にくらべて光合成能力は低く、補償点も呼吸能力も高い、この一時間あたりの光—光合成曲線と各月の平均の日照時間と光の強さの日変化から元気のよい壮葉の一日の相対照度—光合成曲線が各月ごとに計算された。葉の厚さもまた若い葉が展開した後、しだいに増し、この増加は、冬期をのぞいて翌年の6月末まで増え、後しだいに薄くなる。

各器官の呼吸能力は年令が増すにつれて減少するが、3年以上たった桿は桿令に関係なくほぼ一定の値を維持する。これはおもに桿が肥大生長も伸長生長もせず、生きている組織と死んでいる組織の割合が変わらないためであろう。また1年以上の年令の各器官の呼吸能力はいずれも季節によって異なり、雪解後急激に増大し、貯蔵物質の新生器官への転形が最もさかんな6月下旬から7月上旬に最大に達し、後、積雪期まで徐々に低下する。各器官の含水量の変化もまったく呼吸能力の変化と同じ傾向を示す。

各器官の呼吸の Q_{10} は $10 \sim 20^{\circ}$ で $1.9 \sim 2.2$, $3 \sim 10^{\circ}$ で約 2.6 であることがわかった。(東京都立大学理学部生物学教室)

On the Growth Substance Economy before and after Flowering in Each Organ of *Portulaca grandiflora* Hook.

by Takeyosi HORI* and Masaji FUJII*

Received April 5, 1961

Chailakhyan¹⁾ (1938) in his experiments on the *Perilla nankiensis* named the flower initiation hormone and the flowering hormone "florigen." Melchers (1939²⁾) in his experiments on the flowering of annual, biennial, short-day and long-day plants named the flowering hormone "vernalin."

It seems quite probable that the growth substance is related to the phenomenon of elongation since the petals elongate in flowering. Therefore it is of importance to observe the growth substance economy in the petals in order to make clear the mechanism of the opening and closing of flowers.

Various experiments are now in progress on the phenomena of the opening and closing of flowers.

In our experiments was measured the growth substance content in the petals and other organs before and after flowering. The phenomena of the flowering and the wilting of the flowers were also observed.

Materials and Methods

1. *Material*: *Portulaca grandiflora* Hook. was used in our experiments. The seeds were planted in the sand in a green house at 27°-28° in March. The plants were transplanted between May and June. The experiments were carried out from July to August. The petals were collected at 20 and 24 o'clock on the day before flowering and in every 2 hours from 2 o'clock to 24 o'clock on the day of flowering, and at 4 and 8 o'clock on the day after flowering. The ovaries, the leaves and the stems were collected at 6, 12 and 18 o'clock on the day of flowering. The material for dry weight measurement was collected from the separate experimental material, and dry weight per 1 g. fresh material was measured.

2. *Agar block*: Agar block was manufactured by the method mentioned in the Experimental Text-book of Plant Physiology³⁾. That is, the growth substance was extracted with ether from the material, and agar block (2×2×2 mm.³) was made by evaporating the ether and adding agar to the residue.

3. *Avena test*: *Avena* test was used for the bioassay of the growth substance according to the Experimental Text-book of Plant Physiology³⁾, and *Avena* seeds Victory 1) were obtained from Hokkaido in 1958. The curvature was measured by the shadow print. The growth substance content was measured in relation to the IAA concentration and the curvature⁴⁾.

Results and Discussion

1. *Morphological changes in flowering, and the growth substance and the water content in petals.*

The flowers are covered with the calyxes at 20 o'clock on the day before flower-

* Biological Laboratory, Gifu University, Nagara, Gifu, Japan.

ing, and only a small portion of the petals is observed at the tip of the calyxes. This stage continues till 6 o'clock on the day of flowering, and about at 7 o'clock the flower buds suddenly become large and the petals burst open.

The changes that take place between 6 and 10 o'clock are remarkable, and the flowers are in full bloom at 10 o'clock. This state of full bloom continues for 1 or 2 hours, and the flowers begin to wither at 14 o'clock. The morphological changes of the flowers, the growth substance economy and the water content in the petals are as follows. The flower buds gradually become inflated, and the growth substance content increases gradually, too (Fig. 1). The flower buds suddenly begin to burst between 6 and 8 o'clock on the day of flowering. The growth substance content also shows a rapid increase. The growth substance content at 8 o'clock is ten times and that at 10 o'clock is 50 times, as much as that at 6 o'clock. The growth substance content which has reached its maximum at 10 o'clock decreases rapidly after flowering, being at 12 o'clock only one-twelfth of that at 10 o'clock, and after that shows a gradual decrease (Fig. 1). This means that the growth substance content in the petals is at its maximum when the flowers are in full bloom. The changes that take place in the water content have a similar tendency to those in the growth substance content. The water content, which has been increasing gradually till 6 o'clock, shows a rapid increase between 6 and 10 o'clock and decreases gradually between 10 and 18 o'clock, and after that decreases remarkably (Fig. 2).

On the whole, both the water content and the growth substance content increase rapidly when the flowering begins, reach their maximum when the flowers are in full bloom, and decrease rapidly after the flowering has ceased. Though we have not yet

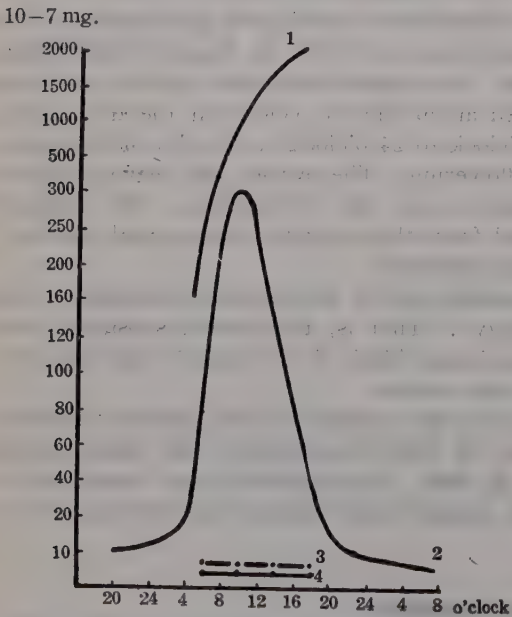


Fig. 1. The growth substance economy in each organ. Ordinate, time of day; the day before flowering, the day of flowering, the day after flowering; abscissa, the growth substance content (mg.) in dry weight 100 mg. 1, ovary; 2, petal; 3, stem; 4, leaf.

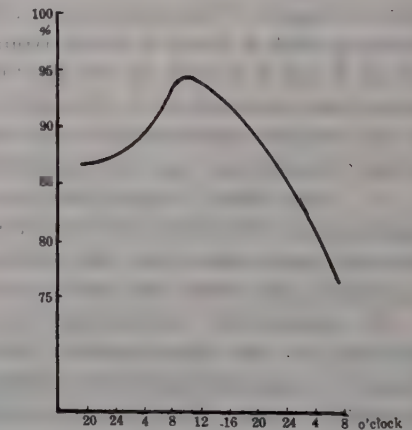


Fig. 2. The water content in the petal. Ordinate, per dry weight 100 mg.; abscissa, time of day; the day before flowering, the day of flowering, the day after flowering.

published this in any written form, in the petals, the tendency as observed in the change of growth substance and water content was also noticed in that of respiration, and glucose and fructose content.

From this fact, it is found that the growth substance is brought into full activity when there are abundant water, glucose and fructose in the petals. Moreover, in order that the flowers may come out, a certain period of time and a certain temperature are required after the differentiation of the flower buds (Reference to this observation is to be found in "An Experiment on Environmental Factors in Flowering" by Hori T., unpublished). And it seems quite probable that tryptophan is accumulated only when a certain amount of heat, light and darkness are allowed for a certain period of time, and that the growth substance is produced with the changes of the composition in the cells of other organs of the flowers.

2. *The growth substance economy in the leaves, stems and ovaries.*

No change is found in the amount of growth substance of the leaves and the stems, even when the growth substance content in the petals is at its maximum at 10 o'clock (Fig. 1). The flowers open even when the leaves are eliminated on the 2nd day before flowering (Reference to this observation is to be found in "An Experiment on Environmental Factors in Flowering" by Hori T., unpublished).

In the normal green plant, auxin is produced in the apical bud or buds and translocated from these buds to the lower portions of the stems. The young expanding leaves may also produce auxin in large amounts, and in many species mature leaves serve as centers of auxin synthesis, although they in general export only a small or negligible quantity of the material⁶).

Experiments were carried out by Söding (1926⁸)) with the flower stalks of *Cardamine*, *Cephalaria*, and some composites and he showed that the auxin-producing organ was, in these cases, the flower or the inflorescence. This was confirmed for *Bellis* by Uylder (1927⁹)).

Therefore it may be concluded that there is no direct relationship between the growth substance in the leaves and that in the petals, and that the growth substance in the petals is produced in the petals themselves which are homologous with the leaves, in the same way that the growth substance is produced in the leaves and the buds.

The growth substance in the ovaries increases continuously (Fig. 1). From this fact it may be inferred that the growth substance increases with growth of the ovaries as the result of pollination, and that there is no direct relationship between the growth substance in the petals and that in the ovaries. Now the growth substance in the ovaries keeps on increasing and the ovaries grow remarkably after the petals have grown up and the growth substance in the petals has reached its maximum. From this we find that the period of growth in each organ is different.

The growth substance increases while the petals and the ovaries are growing, and the growth substance in the petals decreases after the petals have wilted, and so the growth substance economy in each organ is in accordance with the external changes in it.

3. *On the closing of the flowers.*

After 10 o'clock the growth substance, water, respiration, glucose and fructose

in the petals decreases rapidly and the petals cease to elongate. It seems that the increase of the respiration, the consumption of the glucose and fructose, and the decrease of the growth substance in flowering cause the petals to cease to elongate, and one of the causes of wilting may principally be the decrease of water, because the water in the petals decreases, as the supply of water from the roots is intercepted after the formation of the abscission zone⁸⁾, and at the same time water evaporates from the leaves. It may be concluded that the petals wilt more quickly as a large quantity of water evaporates at a high temperature and as glucose in the cells decreases. To sum up, in order to bring light on the subject of the opening of flowers and their closing, studies of the metabolism in the organs and their correlation are required. And so studies are now in process on the carbohydrate, N-compounds and other organic or inorganic compounds and the activities of the respiratory enzymes such as catalase, peroxydase, etc., in various organs of *Portulaca grandiflora* Hook. and the various factors of the environment (temperature, light and water supply etc.).

Summary

The growth substance of the petals, leaves, stems and ovaries before and after flowering in *Portulaca grandiflora* Hook. was measured by the *Avena* test. The results are as follows.

1. The growth substance content in the petals is at its maximum at 10 o'clock when the flowers are in full bloom. Its increase and decrease in the petals before and after flowering are remarkable.
2. The growth substance in the petals is produced in the petals themselves.
3. The water content in the petals is at its maximum at 10 o'clock when the flowers are in full bloom.
4. The growth substance content in the leaves and the stems is constant on the day of flowering.
5. The growth substance content in the ovaries increases on the day of flowering.
6. The order of the growth substance content per unit dry weight is ovary > petal > stem > leaf.

References

- 1) Chailakhyan, Kh., Compt. rend. Acad. Sci. U.S.S.R. 16: 227 (1937), 18: 607 (1938).
- 2) Melchers, G., Ber. dtsch. Bot. Ges. 57: 27 (1939).
- 3) Usami, S., Exp. Text-book of Plant Physiol. Tokyo (Japanese), 318 pp. (1951).
- 4) Hori, T., Sci. Rep. Gifu Univ. 2 (No. 4): 361 (1960).
- 5) Bonner, J., and Galston, W., Principles of Plant Physiol., W. H. Freeman and Co., 357 pp. (1952).
- 6) Söding, H., Jahrb. wiss. Bot. 65: 611 (1926).
- 7) Uldert., I.E., Proc. Kon Akad. Wetensch. Amsterdam 31: 59 (1927).
- 8) Hori, T., Sci. Rep. Gifu Univ. 2 (No. 3): 249 (1959).

摘 要

堀武義, 藤井雅二: マツバボタンの開花・閉花前後の諸器官における生長素の消長
マツバボタンの花卉, 葉, 茎, 子房の生長素をアベナテストによって測定した。その結果は次のようである。

1. 花卉中の生長素は満開時刻の10時に最大となり、その前後の生長素の増減はきわめていちじるしい
2. 花卉中の生長素は葉の中の生長素とは直接関係なく、花卉で作られる。
3. 花卉中の水分含量は満開時刻の10時に最大となる。
4. 葉, 茎の生長素は開花当日では一定である。
5. 子房の生長素は閉花当日では上昇の一途をたどりその量は花卉, 葉, 茎のいずれよりもきわめて多い。
6. 生長素の量は子房 > 花卉 > 茎 > 葉の順である。(岐阜大学生物学教室)

Effect of Temperature and Nutrients on Flower Initiation of *Raphanus sativus* L. in Total Darkness

by Kazuyoshi KIMURA*

Received April 12, 1961

Biennial or winter annual plants are known to require low temperature at the initial stage of plant growth and subsequent high temperature at long day condition for flower initiation. However, evidences are available which show that flower primordia of certain higher plants can be initiated in total darkness¹⁻¹¹). Tashima and Kimura¹⁰) found that flower primordia of *Raphanus* plants were initiated during 40 day cold treatment at 5° in total darkness, visible flower buds were initiated, and some of them even flowered at the end of 120 day cold treatment.

By using aseptic culture method, the present experiments were conducted in order to study the effect of temperature, nutrients and cotyledons on flower initiation in total darkness.

Material and Methods

The material used for the experiment was a variety of *Raphanus sativus* L., Minowase. This variety is highly sensitive to vernalization. Unless otherwise mentioned, the basic culture medium was a modified White's medium containing $\text{Ca}(\text{NO}_3)_2$ 200 mg., MgSO_4 360 mg., Na_2SO_4 200 mg., KNO_3 80 mg., KCl 65 mg., NaH_2PO_4 16.5 mg., MnSO_4 4.5 mg., ZnSO_4 1.5 mg., KI 0.75 mg., Fe-citrate 4 mg., sucrose 50 g., agar 10 g. and distilled water 1000 ml. The test tubes containing about 10 ml. of the culture medium were autoclaved at 1.5 kg./cm.² overpressure for 20 minutes.

Seeds were sterilized by immersing them in 75% alcohol for 5 minutes, in 10% calcium hypochlorite solution for 30 minutes and then in 3% hydrogen peroxide for 30 minutes. Four seeds were sown in each tube. The tubes were wrapped with light-proof paper, kept at 25° for 24 hours and then subjected to various experimental treatments.

The developmental stages of flower primordia are classified in five grades (Fig. 1). Each experiment was repeated two or three times, and almost similar results were obtained. Therefore only typical results are reported in this paper.

Experimental Results

1. *Effect of temperature:* After 24 hour incubation at 25°, the tubes containing germinating seeds were placed under constant temperatures of 0°, 5°, 10°, 15° and 20–25° in total darkness for 120 days (Table 1). The flowering response was maximum at 5°. It decreased with the rising of temperature, and all the plants at 20–25° died at vegetative stage after having developed about 10 leaves. Plants cultured at 0° grew poorly and developed only 5 to 6 leaves. They developed no flower buds at the end of an additional 40 day treatment at 0°. But when these plants were transferred into another 10 day treatment at 25°, they initiated flower buds at the 7.5th node on the average.

* Laboratory of Applied Botany, Faculty of Agriculture, Kyoto University, Kyoto, Japan.

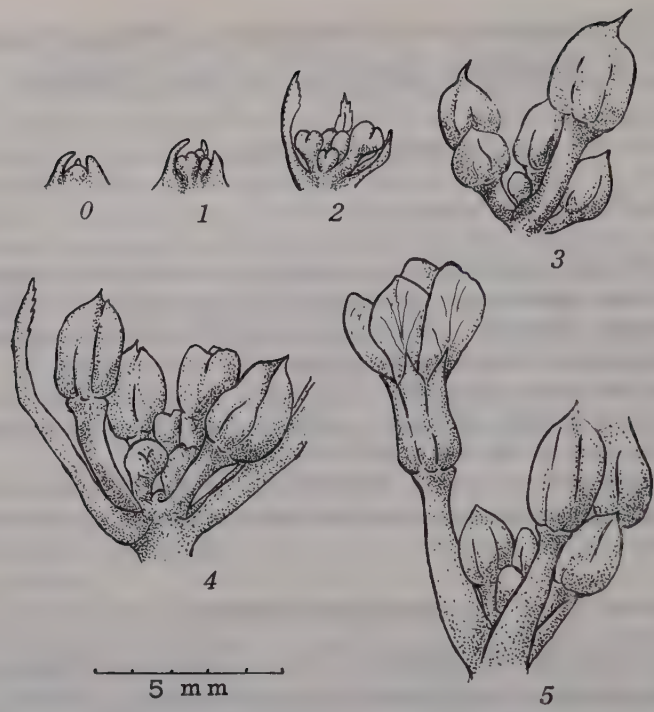


Fig. 1. Developmental stages (0-5) of flower primordia of *Raphanus sativus* L.

- Stage 0 : Vegetative state.
- Stage 1-3: All flower primordia are covered with leaves.
- Stage 4 : Flower buds are exposed from leaves.
- Stage 5 : Open flowers.

Table 1. Effect of temperature on flower initiation in total darkness for 120 days.

Temperature (°C)	No. of plants	Flowering (percent)	Flowering stage	No. of leaves*	Epicotyl length in mm.*	
0	16	0	0	(6.3±0.25)	—	(1.2±0.13)
5	50	96	2.7±0.11	6.6±0.13	4.7±0.14	(4.7±0.42)
10	50	72	2.2±0.20	7.8±0.17	12.0±0.95	(11.5±0.88)
15	20	20	0.5±0.24	8.3±0.22	10.4±0.84	(7.3±1.01)
20—25	19	0	0	(10.7±0.46)	—	(8.8±1.03)

* The number of leaves and the length of epicotyls of plants without flower primordia are enclosed in parentheses.

The length of epicotyls in the flowering plants was 4.7 mm. at 5°, that is, the plants cultured at 5° initiated flower buds without notable elongation of epicotyl.

2. *Effect of sucrose:* The effect of sucrose in the medium on flowering in total darkness was examined with the plants subjected to 5° for 50 days and 120 days. The observation in the latter group was carried out at the end of the cold treatment, and in the former, 30 days after submitting to 20-25° following the 50 day cold treatment.

The result was evaluated from the percentage of flowering plants and from developmental stages of flower primordia.

In 50 day cold treatment, flowering responses were nearly the same over the range of sucrose concentration from 2 to 10 percent, although all the plants cultured on media without sugar remained in the vegetative state and died after having developed about 6 leaves (Table 2). But the plants which were kept at 5° throughout the experiment for 120 days initiated flower primordia without any supply of sucrose and any light treatment (Table 3).

Table 2. Effect of sucrose on flower initiation in total darkness (50 day vernalization).

Percentage of sucrose in media	No. of plants	Flowering (percent)	Flowering stage	No. of leaves
0	15	0	0	(6.7±0.18)*
2	40	98	2.7±0.10	6.6±0.14
5	40	100	3.3±0.13	6.7±0.14
10	28	96	2.8±0.15	7.0±0.15

* Vegetative plants without flower primordia.

Table 3. Flower initiation in total darkness at 5° for 120 days.

Percentage of sucrose	No. of plants	Flowering (percent)	Flowering stage	No. of leaves
0	40	100	2.4±0.13	6.1±0.13
5	30	100	3.1±0.09	6.3±0.13

It is noteworthy that when *Raphanus* plants receive sufficient cold treatment, they produce flower buds in total darkness without any supply of sucrose. This result does not coincide with many reports^{3, 6-11}) which claimed the necessity of sucrose supply for floral initiation in total darkness.

3. *Effect of inorganic nutrients*: In order to examine the effect of nutritive components, the following series of culture medium were used. A, 1% plain agar; MA, minerals+1% agar; SA, 5% sucrose+1% agar; and MSA, minerals+5% sucrose+1% agar.

The plants germinated on the above media were kept in a dark incubator at 5° for 120 days. Control plants were grown under continuous illumination and in total darkness, at 20-25°. Flower primordia were initiated in all the plants kept in the dark at 5° irrespective of the components of culture medium, while at 20-25° no flowering was observed regardless of components or light conditions (Table 4). The flower initiation seems not to depend upon the composition of the culture medium but only on the temperature; however, in the media with sucrose, more advanced stages of flower primordia were observed than in those without sucrose.

From this fact it may be concluded, that the addition of sucrose to the medium affected the floral growth rather than the flower initiation. The substances needed for the latter process seems to be stored in the seed or prepared by the cold treatment under dark condition.

Table 4. Flower initiation after 120 days on the media of different compositions.

Tempera- ture of culture	Light condition	Components of culture**	No. of plants	Flowering (percent)	Flowering stage	No. of leaves***
5°	total darkness	A	18	100	2.4±0.12	6.4±0.21
		MA	20	100	2.1±0.13	6.6±0.18
		SA	23	100	3.5±0.13	6.4±0.17
		MSA	27	100	3.6±0.08	6.3±0.17
20–35°	continuous illumina- tion*	A	15	0	0	(10.5±0.31)
		MA	15	0	0	(13.3±0.49)
		SA	15	0	0	(10.9±0.31)
		MSA	15	0	0	(10.3±0.28)
	total darkness	A	10	0	0	(8.3±0.19)
		MA	10	0	0	(8.3±0.25)
		SA	10	0	0	(9.1±0.28)
		MSA	10	0	0	(11.8±0.47)

* As light source a 100 watt incandescent lamp and two 20 watt day light fluorescent tubes were used, which were fixed in the incubator, 50 cm. above the plants.

** A, 1% plain agar; MA, minerals+1% agar; SA, 5% sucrose+1% agar; MSA, minerals+5% sucrose+1% agar.

*** The number of leaves of plants without flower primordia is enclosed in parentheses.

4. *Effect of the cotyledons*: In order to examine the effect of cotyledon, either one or two of cotyledons of *Raphanus* plants were cut off aseptically 2 days after germination. The treated plants and the intact plants were kept under dark conditions at 5° on the White's culture medium containing 1% agar and 5% sucrose for 50 days and then subjected to darkness at 20–25° (Table 5).

All of the intact plants initiated flower primordia. In the plants with one cotyledon and without any coty-ledon, the percentages of flowering plants were 83% and 63%, respectively. Flowering stage and the number of leaves varied with the number of coty-ledons.

Plants with two, one or no coty-ledons were kept on the medium without sucrose or 5% sucrose at 5° for 120 days (Table 6). On the media containing sucrose, all plants initiated flower primordia and the number of leaves did not vary with the number of

Table 5. Effect of the cotyledons on flower initiation in total darkness (50 day vernalization).

No. of cotyledon	No. of plants	Flowering (percent)	Flowering stage	No. of leaves
0	16	63	1.8±0.35	8.9±0.25
1	18	83	2.3±0.28	8.1±0.21
2	30	100	3.6±0.11	6.5±0.20

Table 6. Effect of cotyledon and sucrose on flower initiation in darkness at 5° for 120 days.

Percentage of sucrose in media	No. of cotyledon	No. of plants	Flowering (percent)	Flowering stage	No. of leaves*
0	0	10	0	0	(5.6±0.28)
	1	10	0	0	(5.7±0.25)
	2	20	100	2.1±0.13	6.6±0.18
5	0	20	100	3.1±0.04	6.8±0.25
	1	25	100	3.2±0.10	6.3±0.14
	2	27	100	3.6±0.08	6.3±0.17

* The number of leaves of plants without flower primordia is enclosed in parentheses.

cotyledons. More advanced stages of flower primordia were found in the plants with two than in those with one or without cotyledons. On the media without sugar, flower primordia were observed on all the intact plants, but not on those with one or no cotyledons.

More detailed experiments were carried out in relation to various combinations of the components of culture medium and the number of cotyledons (Table 7). On A

Table 7. Effect of cotyledon in relation to the components of the culture medium on flower initiation in darkness at 5° for 120 days.

Components of culture media*	No. of cotyledon	No. of plants	Flowering (percent)	Flowering stage	No. of leaves**
A	0	13	0	0	(5.5±0.25)
	1	10	0	0	(6.0±0.29)
	2	17	94	1.3±0.19	6.5±0.12
MA	0	8	0	0	(6.1±0.21)
	1	8	0	0	(6.3±0.18)
	2	19	100	1.6±0.16	6.7±0.14
SA	0	15	100	3.5±0.13	6.1±0.21
	1	15	100	3.4±0.16	6.4±0.21
	2	28	100	3.8±0.11	6.3±0.13
MSA	0	20	100	2.9±0.11	7.1±0.16
	1	20	100	3.1±0.07	6.8±0.18
	2	26	100	3.2±0.09	6.5±0.12

* A, 1% plain agar; MA, minerals+1% agar; SA, 5% sucrose+1% agar; MSA, minerals+5% sucrose+1% agar.

** The number of leaves of plants without flower primordia is enclosed in parentheses.

and MA media, the plants deprived of one or two cotyledons remained entirely vegetative and died after having developed 5-6 leaves, but on SA and MSA media, all the plants initiated flower primordia. The number of cotyledons did not significantly influence the percentage of flowering plants and the number of leaves produced. The flowering stage showed low values on sugarless medium.

When the plants were cultured on the medium containing 5% sucrose at 5° for 120 days, the plants having no cotyledon initiated flower primordia in total darkness. The plants with cotyledons on sugarless medium gave the same result. Sucrose has the same effect on initiation of flower primordia as cotyledons.

Discussion and Conclusion

As regards the flower formation, several theories have been advanced¹²⁻¹⁵). In general, biennial or winter annual plants complete their flowering process under long day conditions at high temperatures following the cold treatments¹⁶). Melchers¹³) believed that in biennial and winter annual plants a hormone, "vernalinal", is produced by low temperature treatment and then another hormone, "florigen", is produced with a long day condition at high temperature.

For flower initiation of *Raphanus sativus*, Tashima reported that the effect of vernalization began to be observed after 5 day treatment at 5-7°, and it reached its maximum after 15 to 20 days. In aseptically culture the first flower primordium under total darkness was differentiated at the same node as that under long days, but under short day conditions it appeared at higher node. The results of Tashima and Kimura's experiment¹⁰) indicated that light and high temperature are not essential to the initiation of flower primordia in this plant; the primordia are already initiated in total darkness during the period of 40 day cold treatment, and at the end of 90 or 120 day treatment, visible flower buds and even open flowers were developed.

In the present experiments in total darkness the flower primordia were induced only by low temperature treatment without warm photoperiod. The flowering response decreased with the rising of temperatures from 5° to 25°. At 20-25°, which is the optimum temperature for vegetative growth of radish, the flowering was not observed even after 120 days. Vegetative growth is strongly retarded under cold condition, but the flowering process of the plants in total darkness was completed under cold condition. Although no morphological changes are recognized at the growing point, the flowering of plants seems to be induced even at 0°, because flowering occurred soon after treatment of the plants at high temperature.

In general, the plant which requires vernalization would bolt accompanied by flower formation; the main axis would begin to elongate to form an inflorescence. In this study, however, the flower primordia can be initiated without elongation of the epicotyl, and the length of epicotyl is decreased at low temperature, though the percentage of flower initiation and the stage of flowering increased. In experiments with wheat (Kimura, unpublished experiment), the author has observed that the stem is capable to elongate without initiating flower primordia by gibberellin application. Konishi¹⁷) observed that the bolting of *Silene* varied with the seasonal changes of temperature. Murneek¹⁸) reported that *Rudbeckia* plants with extremely reduced or inhibited stem (rosettes) were able to produce flowers normally under a certain combination of photoperiods and that stem elongation and flowering were the two separable physiological phenomena. In view of these observations, it may be considered that the stem elongation (bolting) is not always concomitant with the flower

initiation.

Some workers have succeeded in initiating flower primordia in total darkness by a sugar feeding technique^{3,5-11}). They claimed that the addition of sugar to the medium had a physiological significance for flower initiation. The plant in the present experiment, however, initiated flower primordia on a medium containing no sugar (Table 3, 4). This fact seems to indicate that all the materials or their precursors required for flower initiation in darkness are produced in the germinating seed during the cold treatment of sufficient length. The plants themselves may produce sufficient amount of hypothetical flowering substances.

Many investigations on the effect of cotyledon or endosperm on vernalization have been made^{4,9,19,20,21}). When cotyledons were removed, the flower initiation of the plant, kept in total darkness at 5° for 120 days, occurred only on the medium supplied with 5 percent sucrose (Table 6, 7). In floral initiation the roles of cotyledon seem to be replaced by sucrose.

The developmental stage of flower primordia at the end of 120 day culture becomes more advanced with the supply of sucrose or in the presence of the cotyledon; the development of flower primordia may be promoted by sucrose or by the cotyledons. Minerals had a very slight influence on flowering. The dry weight of the plants was greatly affected by the environmental factors, but no clear correlation between dry weight and flowering response was found.

Summary

The flowering behavior of *Raphanus sativus* L. aseptically cultured in total darkness was studied.

1. The plants developed flower buds by 120 day cold treatment in total darkness. The flowering response is maximum at 5° treatment, and it decreases with the rising of the treated temperatures from 5 to 25°. At 20-25° the flowering is not observed even after 120 days.

2. In dark culture at 5° for 120 days, the plants initiate flower primordia even on plain agar medium. All the substances required for flower initiation in darkness seem to be produced in the seed by cold treatment.

3. Plants deprived of cotyledons initiate flower primordia only in the presence of sucrose.

4. Light is not necessary for the flowering during or following the thermal treatment; the combination of long days and high temperature is not essential to the initiation of flower primordia.

I wish to express my deep gratitude to Prof. S. Imamura and Assist. Prof. M. Hamada, Laboratory of Applied Botany in Kyoto University, and to Assist. Prof. Y. Tashima of Kagoshima University for their helpful suggestion and criticism throughout the investigation.

References

- 1) Borgström, G., Bot. Notiser 1939: 830 (1939).
- 2) Fife, J. M., and Price, C., Plant Physiol. 28: 475 (1953).
- 3) Gentcheff, G., and Gustafson, A., Hereditas 26: 250 (1940).
- 4) Haupt, W., Bot. 40: 1 (1952).
- 5) Leopold, A. C., Plant Physiol. 24: 539 (1949).
- 6) Spoehr, H. A., ibid. 17: 379 (1942).
- 7) Sugino, M., Bot. Mag. Tokyo 75: 360 (1957).
- 8) Tashima, Y., Mem. Fac. Agr. Kagoshima Univ. 2: 1 (1956).
- 9) —, ibid. 3: 25 (1957).
- 10) —, and Kimura, K., ibid. 3: 59 (1958).
- 11) —, and Imamura, S., Proc. Jap. Acad. 29: 581 (1953).
- 12) Caj-

lanchan, W. H., *Izd. Akad. nauk USSR, Moscow* (1937) (cited from Murneek¹⁶) 13) Melchers, G., *Ber. dtsch. Bot. Ges.*, **57**: 29 (1939). 14) Napp-Zinn, K., *Planta* **50**: 177 (1957). 15) Purvis, O. N., and Gregory, F. G., *Ann. Bot. N. S.* **16**: 1 (1952). 16) Murneek, A. E., and Whyte, R. O., *Vernalization and Photoperiodism*, Waltham, Mass. U. S. A. (1948). 17) Koniishi, M., *Mem. Coll. Agr. Kyoto Univ.* **71**: 1 (1952). 18) Murneek, A. E., *Bot. Gaz.* **102**: 269 (1940). 19) Gregory, F. G., and Purvis, O. N., *Ann. Bot. N. S.* **2**: 237 (1938). 20) Kojima, H., Yahiro, M., and Inoue, S., *Bot. Mag. Tokyo* **67**: 112 (1954). 21) Yamasaki, Y., *Agr. and Hort.* **19**: 989: (1944).

摘 要

木村和義： 全暗黒条件下におけるダイコンの花芽分化におよぼす温度および養分の影響

全暗黒条件下で、ダイコン(ミノワセ)を無菌培養し、温度、糖、無機塩および子葉の有無が花芽の分化におよぼす影響を調べた。

1. 本植物は全暗黒中でも低温で花芽を分化する。花芽の分化は 5° で、もっともよく、温度が高くなるにしたがって低下し、 $20\sim 25^{\circ}$ ではまったくみられなかった。

2. 5° で 120 日間培養すると、糖および無機塩を含まない純寒天のみの培養基上でも花芽を分化する。糖を含む培養基上では子葉をもたない植物でも花芽を分化するが、糖を含まぬ培養基上では子葉が存在せぬと花芽を分化しない。

3. 低温中では、花芽分化をした植物でも、子葉上茎がほとんど伸長しない。すなわち本植物では花芽形成と茎伸長はかならずしも、あいともなうものではないことがわかる。(京都大学農学部応用植物学研究室)

メタセコイアの緑葉および紅葉中の カロチノイドについて

肥田美知子*・井田 和子*

Michiko HIDA* and Kazuko IDA*: Studies on Carotenoids of Green and
Autumnal Red Leaves of *Metasequoia glyptostroboides*

1961 年 4 月 8 日受付

秋季落葉の前に紅葉したメタセコイアの葉から、塩酸で処理することによって、シアニジン、デルフィニジンを分離することができたが、配糖体であるアントシアニンの分離は不成功に終わった¹⁾。紅葉から分離してきたこれらのアントシアニンはアントシアニンの分解によるものではなく、大部分がロイコアントシアニンの分解によって生じたものである。したがって、メタセコイアの美しい紅葉現象を起こすおもな色素はアントシアニン系のものとはほとんど関係がなく、ヒノキ、スギなどで知られている²⁾カロチノイド系の色素によることが考えられる。

また、秋に起こる葉の黄化は緑葉中のクロロフィルの分解によって、共存していたカロチノイドが目につくようになることが多い。メタセコイアの紅葉の場合にも、緑葉のときからあったカロチノイドがクロロフィルの分解の結果、表面にあらわれてきたものか、あるいは別なカロチノイドが新たに加わったものかを知るために、葉の開いたころから落葉までの各月に材料をとり、カロチノイドの種類および量の季節的变化をカラムクロマトグラフィー、および分光光度計によってしらべた。その結果としてメタセコイアの紅葉には特殊な桃色色素が存在することがわかったので、ここにその概要を報告する。

材料および方法

材料は大阪女子大学の庭のメタセコイアから葉の

開く4月下旬を最初にして12月20日ごろ、葉が全部落ちてしまうまでの間、4月を除いて毎月3回以上、12月の紅葉期には毎週1回づつ葉を採集し、林孝三著、植物色素実験法³⁾に記載の方法によって、カロチノイドを分離し、各種溶剤にとかし、分光光度計にかけて吸収波長をしらべると同時に、呈色反応をみてカロチノイドの種類を同定した。また、最大吸収波長においての吸収度の増減から葉中のカロチノイド量の増減を推定した。

なお、比較のために市販(第一化学薬品株式会社)の β -カロチンについても、吸収波長、Rf値などを測定した。

1. 抽出

生鮮材料を5g.とって細切し、これに石油ベンジン 20 ml., メタノール 20 ml.を加え、ときどき振りながら25~30°に15分間放置後、液を傾斜し、残渣に再び石油ベンジン 20 ml., メタノール 20 ml.を加えて抽出を行なう。このような操作を4回繰り返し、傾斜した液を全部一緒にすると合計160 ml.の緑色の抽出液が得られる。

2. ベンジン層の精製

(1)によって得た160 ml.の抽出液を円筒分液漏斗に移し、よく振った後、静置するとベンジン層(上部)とメタノール層(下部)に分離してくる。この上部層、すなわちベンジン層は下記の、メタノール層は(3)の方法によってそれぞれ精製する。

円筒分液漏斗に残ったベンジン層に90%メタノールを20 ml.加え、よく振り静置後メタノール層を捨てる。この操作を4回繰り返す。90%メタノール

* Biological Laboratory of Osaka Women's University, Osaka Japan. 大阪女子大学生物学教室

で洗ったベンジン層を三角フラスコに移し、5%エタノール性 KOH (96%エタノール 100 ml. に KOH 5 g. をといたもの) を 50 ml. 加え、よく振りコルク栓をし、40° の恒温器中に 3 時間放置する。この操作によりエステル型のキサントフィル類が遊離型になる。

けん化を終わった液を再び円筒分液漏斗に移し、10 ml. の蒸留水を加えてよく振り静置するとベンジン層が上部に分離してくる。このベンジン層をとり、これに 90% メタノールを約 10 ml. 加え、よく振ってベンジン層を洗う。洗液が着色しなくなるまで同じ操作を繰り返す。その後水でよく洗い、アルコール分を取り除いたベンジン層をカラムクロマトグラフィの試料とする。

3. メタノール層の精製

(1) の抽出液から下部のメタノール層を円筒分液漏斗に移し、石油ベンジン 20 ml. を加えよく振り、メタノール層を石油ベンジンでよく洗う。この洗液は捨て、再び 20 ml. の石油ベンジンを加えて洗う。この操作を 4 回繰り返し、メタノール層にとけていた少量のカロチン、リコピン、キサントフィルのエステルを完全に除く。洗ったメタノール層を三角フラスコに移し、2 N-NaOH 50 ml. を加え、よく振った後コルク栓をし、3 時間室温に放置する。11 月中旬以後は室温が低いので 30° の恒温器内に入れた。3 時間後、液を円筒分液漏斗に移し、30 ml. のベンジンと 75 ml. の蒸留水を加え、よく振って静置すると液は 2 層に分かれるから、上部のベンジン層だけをこして下層は捨て去る。残った黄色のベンジン層はアルコールが完全になくなるまで水で洗う。このようにして得られた色素のベンジン溶液をカラムクロマトグラフィの試料とした。

4. 色素の単離

上記の方法で得られた色素のベンジン溶液には 2 種以上のカロチノイドが含まれているから、これをそれぞれ適当な吸着塔を通して単離し、単離してきたものを各種溶剤にとかし分光光度計にかける試料および呈色反応をみる試料とした。

a. ベンジン層より分離した色素液の場合

市販のクロマト用活性アルミナを 3 時間以上ガスバーナーで灼熱し、デシケーター中で放冷したものをガラス管に詰め、吸着塔とした。この吸着塔を石油ベンジンで湿らせたのち、色素液 5 ml. を注ぎ、

その後石油ベンジンで展開すると、上部より 5—10 mm. のところに橙色帯がとどまった。その後カラムを取り出し、この橙色帯を切りとり各種溶剤にとかす。

b. メタノール層より分離した色素液の場合

150° 乾燥器に入れた市販の炭酸カルシウムをデシケーター中で放冷しこれを吸着塔にした。湿った吸着塔に黄色色素のベンジン溶液を 5 ml. 注ぐと上部に黄色の帯ができるが、これを石油ベンジンで展開すると、帯は上部と下部の黄色の二帯に分離する。特に下部の黄色色素はすみやかに流出するため、後に用いる溶剤がベンジン以外のものでは下部にとどめるよう展開を加減した。この 2 つの色素帯をそれぞれ吸着塔から取り、各種溶剤にとかした。

なお、紅葉から抽出した色素液では 2 つの黄色帯のほか、その間に桃色の色素帯が分離してきた。この場合、桃色帯はたいてい下部黄色帯と重なりあっているため、2 度吸着塔を通した。すなわち、最初の吸着塔では上部の黄色帯だけを残り、下部の 2 帯は全部流し出し、この液を再び新しい吸着塔にかけ展開し、桃色、黄色の 2 帯を得た。

以上吸着塔から単離した色素は各種溶剤、すなわち、ヘキサン、石油ベンジン、二硫化炭素およびクロロホルムで、クロロホルム以外はすべて 1% エタノール含有のものを用い、これに色素をとかし、日立分光光度計 EPU-2 型で吸収波長をしらべると同時に、各種の溶剤に対する溶解度、溶液の色、カロチノイドの種々の呈色反応をしらべ、それらの結果を総合してカロチノイドの種類を決定した。

5. 呈色反応

a. 濃硫酸による呈色反応：吸着塔から単離した色素をクロロホルムにとかし、少量を試験管にとり濃硫酸を器壁に沿って入れる。

b. 濃塩酸による呈色反応：色素のエーテル溶液に濃塩酸を加え、少量のフェノールを加えて振る。

c. 三塩化アンチモンによる呈色：色素のクロロホルム溶液に三塩化アンチモンのクロロホルム飽和液を加える。

6. ペーパークロマトグラフィによる分離

ベンジン層、メタノール層の精製液を試料とし、東洋ろ紙 No. 50 を用いて展開した。展開剤としては石油エーテル、ベンゼン、石油エーテル：ベンゼン

ン (1:1 v/v), メタノール:エタノール (1:1 v/v) を用いた。これによって分離してくる色素の Rf 値を測定し、判定の資料とした。

実験結果

1. メタセコイアの葉中のカロチノイド
メタセコイアは落葉性の針葉樹で4月下旬に冬芽が開き、柔らかい淡緑色の葉を出す。この葉はしだいに緑色を増し7、8月ころには濃緑色になる。10月下旬には先端が枯れたような赤褐色になりはじめ、日がたつにつれて赤褐色の部分が広がり、12月上旬には全葉が美しい赤褐色になる。この色も一般の広葉樹の紅葉と同じように、年により赤色の勝った褐色のときとよくれた赤褐色の場合がある。これはもちろん気候条件の違いによるものと思われる。こんなに色の変化する葉を4月から落葉まで毎月採集し、そのなかに含まれているカロチノイドをしらべた結果、緑葉のなかにはベンジン層に溶出し、アルミナ吸着塔に吸着するカロチン系の橙色色素と、メタノール層に溶け、炭酸カルシウム吸着塔に吸着する2種のキサントフィル系の黄色色素が含まれていることがわかった。また、11月中旬以後の紅葉した葉からは、これら3種の色素のほか、炭酸カルシウムの2種の黄色色素の帯の間に吸着する桃色の色素が分離してきた。
このように分離してきた4種について呈色反応を行なったが、いずれもカロチノイドの反応を示した。ただ、いずれの場合も色素が微量なために反応

が弱く、特に三塩化アンチモンで反応した溶液を分光光度計にかけたが、好ましい結果は得られなかった。

a. 抽出液のベンジン層に溶出し、アルミナ吸着塔で分離してきた色素
抽出液のベンジン層にはクロロフィル、エステル型キサントフィル、炭化水素系カロチノイドが溶出するが、これをけん化精製すると炭化水素系のカロチノイド以外の色素は除かれる。この精製液をアルミナ吸着塔を通し、ベンジンで展開すると橙色色素が原点よりやや下がったところに吸着した。これを各種溶剤にとかし、分光光度計で吸収波長を測定した結果は Table 1 のとおりで、二硫化炭素およびクロロホルム、ヘキサンでは α -カロチンによく一致するが、石油ベンジンでは α -カロチンならば 447.5 m μ および 478 m μ にあるべき吸収が本実験では 450 m μ , 477 m μ になっている。しかし実際においては 447.5 m μ から 451 m μ の間のいずれかに、また、475 m μ から 478 m μ の間のいずれかに吸収の山があるので、頻度からいって最も高いのは Table 1 の波長になることを意味している。文献の値と測定値とのこの程度の差は、市販の β -カロチンについてもみられるもので、誤差の範囲と考えてよい。また、このカロチンの吸収波長は市販の β -カロチンの値とは違っているし、このカロチンと市販の β -カロチンとの混合液は石油エーテルを展開液として行なつたペーパークロマトグラムで明らかに2種類の橙黄色色素に分離してきた。すなわち、一つは

Table 1. Leaf carotenoids of *Metasequoia glyptostroboides*

Band	Solvent	Light petroleum		CS ₂		Chloroform		Hexane		Decided name
		Color of solution	A. S. M.	Color of solution	A. S. M.	Color of solution	A. S. M.	Color of solution	A. S. M.	
Al ₂ O ₃ column	main band	Yo	425 450 477	O	450 477 509	OY	460 485	Y	420 445 475	α -Carotene
	Upper band	Y	420 443 472	OY	445 469 500	Yo	435 452 481	Yo	420 440 470	Violaxanthin
	Lower band	Y	425 447.5 477	O	445 475 508	Yo	428 455 487	Y	420 445 475	Lutein
CaCO ₃ column	Pink band	O	451 477	P	480 507	PO	460 485	O	450 475	Undecided

A.S.M., absorption spectrum maxima(m μ); Y, Yellow; Yo, Orange Yellow; YO, Yellow Orange; O, Orange; P, Pink; PO, Pinkish Orange.

Rf 値が 0.99 で他は 0.95 を示した。このようなことを総合して今回分離してきた色素は α -カロチンと判定した。

b. 抽出液のメタノール層に溶出し、炭酸カルシウム吸着塔で分離してきた 2 種の黄色色素

抽出液のメタノール層にはキサントフィル類とクロロフィルとの遊離型のものが溶出して来るが、精製によりクロロフィルは除かれ、キサントフィル類だけとなる。これの石油ベンジン溶液を炭酸カルシウムの吸着塔を通し、石油ベンジンで展開すると原点より少し下がったところに淡黄色の帯が、さらに下部にやや橙色がかった黄色の帯が分離してきた。この 2 種の色素の各種溶剤中での吸収波長は Table 1 のようで、この価から上部のキサントフィルはビオラキサンチンで、下部のものはルテインであることが判明した。

c. 抽出液のメタノール層に溶出し、炭酸カルシウム吸着塔に吸着する桃色色素

11 月中旬以後の紅葉した葉から得た抽出液のメタノール層からの精製液を炭酸カルシウムの吸着塔を通すと前記の 2 種の黄色色素の帯と帯の間に桃色色素が吸着した。これの吸収波長 (Table 1) からみれば α -カロチンに近いが、上記の方法で分離すれば α -カロチンならばベンジン層に溶出してこなければならず、その各種溶剤中での色も α -カロチンとは異なっている。また吸収曲線をみても α -カロチンでは短かい波長のほうに最大吸収があるのに、この場合は長い波長のほうが最大吸収を示す (Fig. 1)。この吸収曲線の形からみればプロリコピン、プロ- α -カロチンなどに類似するが、その吸収波長は著し

くいずれとも異なっている。その他のカロチノイドとも吸収波長や、吸収曲線の形を比較検討したが、該当する色素が見いだせず、したがって今回の実験ではカロチノイド名を決定することはできなかった。

2. カロチノイドの季節的变化

葉が開いてから落ちるまでの全期間にわたって α -カロチン、ビオラキサンチン、ルテインが認められたが、その季節的变化をグラフにすれば Fig. 2 のようになる。ここでは各月の吸収度の変化から、その季節による量的変化を推定した。開いて間もない若葉では 3 つのカロチノイドのうち α -カロチンが優位で、他の 2 つのカロチノイドは比較的少ない。 α -カロチンの量は日がたつにつれて増し、5 月には最高値を示すと同時に、3 つのカロチノイドの最高でもある。 α -カロチンはこれを最高にして、しだいに減少し、7 月には谷を示す。 α -カロチンが減少しはじめるころからキサントフィル類の量が増し、いずれも 6 月に最高値を示し、やがて減少していくが、その減り方は α -カロチンほど急激ではない。そしてビオラキサンチンでは 8 月に、ルテインでは 9 月に谷があらわれ、その後やや上昇して前者は 11 月より、後者は 10 月から漸次減少していく。7 月に谷を示した α -カロチンは 8 月に再び量を増し、その後急に減少する。上記の 3 つのカロチノイドが減少しはじめた 11 月中旬には葉の外観はなかば紅葉し、このころからメタノール層に桃色色素が溶出してくる。その後紅葉が進むにつれて、この色素はその量をまし、12 月中旬ころの落葉時には急に減少す

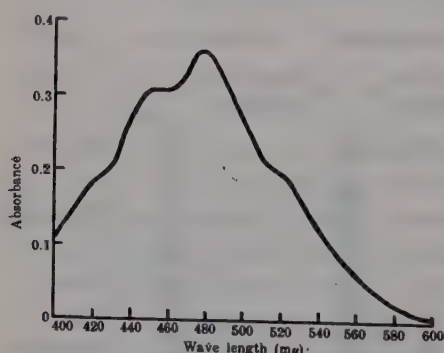


Fig. 1. The absorption spectrum of pink carotenoid from autumnal leaves of *Metasequoia* (in light petrolium).

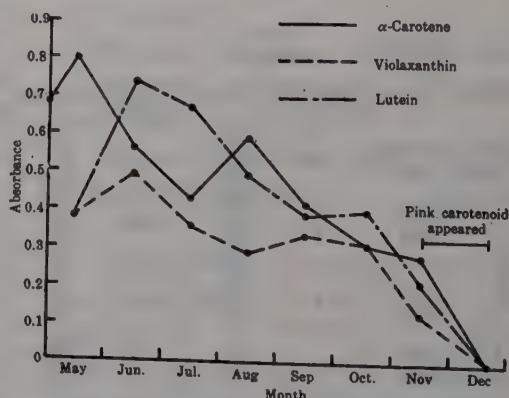


Fig. 2. Variation of carotenoid content in *Metasequoia* leaves.

る。

なお、メタセコイアの含水量は 11 月のはじめから急に減少するため 5g. の試料中のカロチノイドの量は多いのが当然だが、事実は反対に急減している。このグラフでは含水量の減少は考慮していないから、これを入れればさらに急激な減少を示すことになる。

考 察

1. 葉中のカロチノイドの種類

緑葉のなかにはクロロフィルのほかにカロチノイドが存在することは古くから知られていた。すなわち、R. Willstätter (1913) がクロロフィルの研究のときに緑葉中にはカロチンとキサントフィルの 2 種のカロチノイドが含まれていることを明らかにしたが、その後の Strain (1938) などの研究によって緑葉中には狭義のキサントフィル、すなわち、ルテインのほかにビオラキササンチン、ゼアキササンチンなどが存在する場合のあることを見いだした。また、カロチンについては一般には α -カロチンと β -カロチンを含み^{4,5,6)}、多くの植物では β -カロチンが最も普通で、それに少量の α -カロチンを伴っているが、 α -カロチンが単独に存在するものはまれで、ただ Formosa tea plant の緑葉において単独に存在することが知られている⁷⁾。ところが今回の研究では吸収波長やペーパークロマトグラムの Rf 値などから判定して、葉から分離したカロチンは α -カロチンと同定したが、この判断からすればメタセコイアの葉中のカロチンは α -カロチンだけか、もし β -カロチンがあってもごく少量で、少なくとも α -カロチンが優位であると考えられる。最近、京都大学薬学部の高橋三雄博士はメタセコイアの落葉中には α および β -カロチンのあることを報じている⁷⁾が、著者らが β -カロチンを確認できなかったのは少量のためと思われ、したがってメタセコイアの葉はカロチンに関してはまれなタイプの葉ということができよう。

キサントフィル類に関しては炭酸カルシウムの吸着塔の上部に吸着した色素はビオラキササンチンに、下部に吸着したものはルテインにそれぞれ吸収波長も一致するので、メタセコイアの場合も一般植物と同様にこの 2 種のキサントフィルのあることを確認した。

紅葉からは上記の 3 種のカロチノイドのほかに、桃色のカロチノイドが分離してきたが、文献ではこれに該当する色素がない。Monteverde & Lubimenko の研究⁹⁾で冬季、裸子植物のあるもの、たとえば *Thuja virginica*, *Taxus baccata*, *Cupressus naitnockii* などにロドキササンチンが含まれていることを報じているが、分離した桃色色素はロドキササンチンとは吸収波長が異なり、同一物とは考えられない。また、既述のように吸収曲線はプロリコピンやプロト-カロチンに似ているが、これとも異なっているし、最近、下等植物において見つかっている種々のカロチノイドとも一致しない⁸⁾。このように今回の実験ではこの色素がなんであるかは決定できなかったが、明らかにメタセコイアの紅葉現象に関し主役をなしている色素である。その後紅葉したスギからも非常に微量であるが分離することができたし、まだ抽出して検討していないが、石油エーテル展開のペーパークロマトグラムではメタセコイア以外に、イヌスギ (*Glyptostrobus pensilis*)、ヌマスギ (*Taxodium distichum*)、セカイヤメスギ (*Sequoia sempervirens*)、セカイヤオスギ (*Sequoiadendron giganteum*) その他数種の紅葉した針葉樹からメタセコイアと同様に桃色色素が原点の近くに分離してきた。これらの桃色色素が、メタセコイアのもと同一如うかは目下検討中であるが、Rf 値から考えてたぶん同一物と思われる。同一物であれば、この色素が針葉樹の紅葉の主役をなしている色素と考えられよう。

2. カロチノイドの季節的变化

一般に若葉にはカロチンが多く成熟に伴い減少し⁶⁾、キサントフィル類は秋季葉が紅葉するころには酸化的に分解するものと考えられていた⁷⁾が、今回の研究によって明らかに若葉にはカロチンが多く、このころにはキサントフィル類は僅少で、カラムクロマトグラフィーで 2 種に分離することができなかったが、その後葉が成熟するにつれてカロチン量は少なくなるが、途中 7 月に著しく減り、8 月に再び上昇しグラフでは 7 月に谷を作る。このことはキサントフィル類にもいえることで、カロチンにおくられて最大量に達した 2 種のキサントフィル類は、ビオラキササンチンは 8 月に、ルテインは 9 月にそれぞれ谷を作っている。このように谷を作る時期がずれていることは、カロチノイドの生理的役割と考えあ

わせ興味のあることである⁹⁾。また、減少のしかたはカロチンでは比較的急激に減少するが、キサントフィル類はカロチンよりゆるやかに減り、特にピオラキサントフェンは全体的に変化はゆるやかである。

桃色素の出現はカロチンおよびキサントフィル類の減少した 11 月中旬ころからで、従来黄葉においてはクロロフィルの分解により先から含まれていたカロチノイドが眼につくようになるものと考えられていたが、少なくともメタセコイアの紅葉では既存のカロチノイドによるものではなく、新しくできてきた桃色のカロチノイドによるものであることがわかった。さきに著者の一人は針葉樹の紅葉からアントシアニジンを分離した¹⁾が、これはそのときの考

察のように、アントシアニンから生じたものより、むしろロイコアントシアニンの分解によって生じたもので、メタセコイアの紅葉の色素はアントシアニン系のものでなく、桃色のカロチノイドによるものである。このことは他の紅葉する針葉樹についてもいわれることのように目下研究中である。

終りにのぞみ、終始ご懇切なご指導ならびにご助言をいただいた大阪市立大学理学部三木茂博士に心からの感謝を捧げるとともに、含水量測定のをとられた当教室の小野、原田両嬢に厚く御礼申し上げる。

文 献

- 1) 肥田美知子, 植雑 71: 425 (1958).
- 2) 三好 学, 植物学講義上巻 (1924).
- 3) 林 孝三, 植物色素実験法 (1954).
- 4) 服部静夫, 植物色素 (1942).
- 5) Bonner, J., Plant Biochemistry (1950).
- 6) Goodwin, T. W., The Comparative Biochemistry of the Carotenoids (1952).
- 7) 刈米達夫, 文部省科学研究総合研究第 3 集 (1961).
- 8) Green, J., Nature 183: 56 (1959).
- 9) Blass, U., Anderson J. M., and Calvin, M., Plant physiol. 34: 329 (1959).

Summary

We have been investigating the autumnal red leaves of the conifers.

Supposing that coniferous colored leaves contain the anthocyanins, we studied them by means of paper chromatography. However, we could not isolate any anthocyanins, but some anthocyanidins. This fact suggested that these anthocyanidins almost came from leucoanthocyanins, and the coloration of autumnal red leaves might not be due to the presence of anthocyanin but of carotenoid.

In this paper we have studied about carotenoids of *Metasequoia's* leaves. Sampling of materials has been made every month from April to December. Determination of isolated carotenoids has been carried out by the column chromatography and the spectrophotometry. The results are summarized as follows:

1. Green leaves of *Metasequoia* contain three carotenoids: α -carotene, violaxanthin and lutein.
2. The maximum of carotenoid content was found between spring and summer as shown in Fig. 2. After that, carotenoid content decreased gradually, each content-curve having a characteristic concave curve at some period: α -carotene in July, violaxanthin in August and lutein in September. When leaves fell all carotenoids showed minimum contents.
3. In the autumnal red leaves of *Metasequoia* there were found three yellow carotenoids and one pink carotenoid. The former three were α -carotene, violaxanthin and lutein being the same as in green leaves. The latter pink carotenoid showed absorption maxima at 451 m μ and 477 m μ in light petroleum. This pink carotenoid is a main pigment of red leaves of *Metasequoia* in autumn, and it bears a striking resemblance to the pigment of other coniferous autumnal red leaves. We have not, however, been able to identify this carotenoid by this time.

ミズバショウ花穂の呼吸にみられる青酸促進について

桑山弥寿男*・庄 貞行**・宇佐美正一郎**

Yasuo KUWAYAMA*, Sadayuki SHO** and Shoichiro USAMI**: Cyanide-Stimulation of *Lysichiton* Spadix Respiration.

1961 年 3 月 23 日受付

多くのサトイモ科植物の花穂および花穂からとったミトコンドリアが、青酸不感受性の呼吸をしめすことについては、いくつかの報告^{1,2,3)}がある。ミズバショウの花穂も青酸抵抗性の呼吸をしめすことを著者らは前に報告した⁴⁾が、この花穂切片の呼吸は青酸により阻害を受けないだけでなく、かえって 50% も促進される。チトクローム酸化酵素が青酸により阻害されることはよく知られた事実である。サトイモ科植物 *Arum*, *Symplocarpus* の花穂ミトコンドリアは、チトクロームおよびチトクローム酸化酵素を含むことが知られている^{5,6)}が、ミズバショウの花穂ミトコンドリアについても分光学的観察から同様のことが確かめられている⁴⁾。チトクローム酸化酵素をもつ組織の呼吸が青酸阻害を受けない現象の機作については側経路 (Alternate pathway) 説、酸化酵素過剰 (Excess oxidase) 説などが提出されている⁷⁾が、これらの説では青酸による促進現象を説明できない。青酸による呼吸促進現象は成熟した葉についても報告されている⁸⁾が、この場合は重金属の蓄積が呼吸抑制をもたらしており、青酸はそれを取り除くものと推察されている。

われわれは、ミズバショウ花穂ミトコンドリアの自家呼吸が組織内に存在するなんらかの有機物質によって抑制されており、青酸添加はその抑制を除去

するものであることを示す知見を得たので、二、三の関連実験の結果とともにここに報告する。

材料と方法

北海道各地から 1960 年 4~6 月に採集したミズバショウ (*Lysichiton camtschatcense* Schott) の花穂からミトコンドリアを分画した。開花前の発育時期にあるものが高い呼吸活性と青酸による最大の呼吸促進を示す⁴⁾ため、この時期のものを使用した。花穂はすべて花序軸を西洋かみそりで取り除いたものを使用した。花穂 100 g. を 0.25 M. 蔗糖, 0.02 M. 磷酸緩衝液 (pH 7.0), 0.001 M. フッ化ナトリウム, 1 mg./1 ml. 卵白アルブミンの混合液 100 ml. および少量の石英砂とともに氷冷しながら乳鉢で磨砕し、ガーゼで濾過した。濾液を 3,500 回転 5 分間の遠心分離にかけ、石英砂、花粉袋、磨砕されない花粉などを除去した。上澄液をさらに 10,000 回転 15 分間遠心分離して淡黄色のミトコンドリア分画を得た。ミトコンドリアはヤヌスグリーン B によって染色されることを確かめた。この沈殿を少量の磨砕用混合液に懸濁し、10,000 回転 15 分間の遠心分離にかけて洗滌したものをミトコンドリア標品として使用した。分画操作はすべて低温下で行なった。

ミズバショウの花穂は西洋かみそりで約 0.2 mm. の厚さ (めしべ、花粉袋などを含む) の切片とし、サトウダイコンの葉およびタバコの葉はハサミで約 1 mm. × 10 mm. の切片として使用した。

組織を生量と同量の水とともに 90° で 30 分間加熱後すりつぶし、さらに上と同量の水を追加、90° で 30 分間加熱後ガーゼで濾過、濾液を 3,000 回転

*Laboratory of Domestic Science, Iwamizawa Branch, Hokkaido Gakugei University, Iwamizawa, Japan. 北海道学芸大学岩見沢分校家政学教室。 **Department of Botany, Faculty of Science, Hokkaido University, Sapporo, Japan. 北海道大学理学部植物学教室。

10分間の遠心分離にかけて得られる上澄液を組織の熱抽出液とした。熱抽出液の灰は小型の磁製のつぼを用いてつくり、水を加えてもと同容の懸濁液にして使用した。塩化物の生成をさけるために、灰を溶解させるさい塩酸は使用しなかった。

ウシ心筋のコハク酸酸化酵素粗標品の調製はつぎのごとく行なった。新鮮な心筋を磨砕し、10倍量の氷冷水とともに10分間よく攪拌してからガーゼで充分しぼる。この操作を3回くりかえす。この洗滌された組織磨砕物を密閉容器中に凍結保存する。凍結状態のまま組織磨砕物を薄片に切り、その2g.に0.1 M. 磷酸緩衝液 (pH 7.0) 6 ml. を加え、氷冷ガラスホモジナイザーで均一化したものを酵素標品とした。

酸素吸収量の測定にはワールブルク検圧計を用いた。青酸添加実験のさいには容器副室に青酸カリと苛性カリの混液を入れ、主室中の青酸濃度を一定に保たせた。

結果と考察

ミズバショウのミトコンドリアは非常に不安定である。冷蔵しても調製後2時間でその活性は半減する。失活を防ぐため ATP や Mg^{++} を加えても効果は認められなかった。Table 1. にミトコンドリアの自家呼吸におよぼす花穂の熱抽出液の影響を示したが、明らかに呼吸抑制が認められる。青酸添加の影響は抽出液無添加の場合には皆無であるが、抽出液添加の場合には明白に促進的であり、完全に自家呼吸が回復される。この事実は抽出液中にミトコン

ドリアの自家呼吸を抑制する成分の存在すること、しかも青酸添加によってこの成分の呼吸抑制作用は除去されることを示す。熱抽出液を用いているのだから、呼吸抑制が酵素作用によるとは考えられない。

Arum のミトコンドリアは TCA サイクルの有機酸をよく酸化する⁹⁾と報告されているが、ミズバショウのミトコンドリアにコハク酸、あるいはリンゴ酸を呼吸基質として与えた場合、どの場合にも非常に弱い酸素吸収促進しか見られなかった。熱抽出液はミズバショウのミトコンドリアのコハク酸酸化を明らかに抑制したが、青酸添加による回復の現象は認められない。自家呼吸とコハク酸酸化とを比べた場合、青酸の影響がこのように異なっていることは、自家呼吸における主な基質がコハク酸ではないことを示唆する。

ミズバショウのミトコンドリアのコハク酸酸化能が弱いので、牛の心筋から典型的なコハク酸酸化酵素系を抽出し、その酸化能に対するミズバショウ熱抽出液の影響について実験した (Table 2)。熱抽出

Table 2. Effects of boiled extract of *Lysichiton spadix* and of cyanide on the activity of succinoxidase from beef heart. Reaction medium contained 0.04 M. phosphate buffer (pH 7.0), 0.05 M. potassium succinate, and 0.3 ml. enzyme suspension. 0.001 M. HCN and/or boiled extract were added as indicated. Final volume, 2.0 ml. Temperature 30°. Gas exchange measured for one hour.

Extract	Succinate oxidation		Inhibition	
	+ HCN		by extract	by extract plus HCN
ml.	O ₂ μ l.	O ₂ μ l.	%	%
0	144	0		100
0.0002	143	0	0	100
0.002	131	0	10	100
0.005	85	0	40	100
0.01	71	0	50	100
0.02	30	0	80	100
0.30	16	1	90	100
0.45	23	17	80	90
0.60	22	16	90	90
0.75	21	13	90	90
1.20*	26	0	80	100
2.40*	0	0	100	—

*Concentrated extract used.

Table 1. Effects of boiled extract of *Lysichiton spadix* and of cyanide on the oxygen uptake of mitochondria from the spadix. Reaction medium contained 0.04 M. phosphate buffer (pH 7.0) and 0.7 ml. mitochondrial suspension (dry weight 76.4 mg.). 0.001 M. HCN and/or 0.5 ml. boiled extract were added as indicated. Final volume, 2.0 ml. Temperature 30°. Gas exchange measured for 40 min.

	O ₂ uptake	Inhibition (—) or stimulation (+)
	μ l.	%
Endogenous oxidation	24.4	
+ HCN	25.4	+ 4
+ Extract	14.1	—42
+ Extract + HCN	26.3	+ 8

液を加えることにより明らかな抑制がみられ、しかも青酸と抽出液との作用はある程度相互に拮抗的であるようだ。腐敗細菌 *Proteus vulgaris* を音波破碎して得られる無細胞抽出液のコハク酸酸化の場合にも、青酸阻害度はミズバショウ抽出液添加によって低下させられた。

心筋コハク酸酸化酵素系におよぼすこのような影響がミズバショウの花穂の抽出液に特有のものかどうかをしらべるために、タバコの葉とサトウダイコンの葉の抽出液について同様の実験を試みた。まず青酸は Table 3. に示したようにタバコの葉の呼吸に対しては無影響であり、サトウダイコンの葉の呼吸に対しては約 50 % の阻害を与える。つぎにタバコおよびサトウダイコンの葉から、ミズバショウの花穂の場合と同様な方法で作った抽出液が、心筋コハク酸酸化酵素系におよぼす影響をしらべた。Table 4. に見られるように、タバコおよびサトウダイコンの葉の抽出液は、ミズバショウの花穂の抽出液の場合と比較して、その程度は弱いながら、同様な抑制をひきおこした。ただし青酸阻害との間に拮抗現象をあらわさないことが重要な相違点としてあげられる。すなわち、青酸との相互作用はミズバショウ花穂の抽出液に特有の現象であって、この抽出液はおそらく青酸となんらかの結合物をつくりうる成分を含有するものと考えられる。

上記の植物組織の抽出液中には、それぞれコハク酸酸化を抑制する成分が含まれていることは明らか

Table 3. Effect of cyanide on the oxygen uptake of sliced plant tissues. Reaction medium contained 0.04 M. phosphate buffer (pH 7.0) and sliced plant tissues (fresh weight 100 mg.). 0.001 M. HCN was added as indicated. Final volume, 2.0 ml. Temperature 30°. Gas exchange measured for one hour.

Tissues	Endogenous oxidation	+ HCN	Inhibition (-) or stimulation (+)
	O ₂ μ l.	O ₂ μ l.	%
<i>Lysichiton</i> spadix	107	157	+50
Tobacco leaf	34	35	\pm 0
Beet leaf	51	27	-50

であるが、このものが有機物か無機物かを検するために抽出液を灰にしたもののつち効果をしらべた。

Table 5. に示したように、灰分は抽出液そのものに比し軽度の抑制作用をあらわした。ミズバショウの抽出液では灰化による抑制度の低下が著しく、しかもその灰分は青酸阻害度を減少させない。このことは、植物組織中にはコハク酸酸化酵素系を阻害するけれども、青酸とは反応しない、ある種の無機物が含まれていることを示している。

以上の結果から、ミズバショウ花穂では組織中のある種の成分（おそらく有機物）がミトコンドリアの酸化能を抑制しているが、青酸添加によりその抑制作用がとり除かれる結果、呼吸の促進現象がみられるものと推察される。この青酸促進を受ける呼吸の

Table 4. Effects of boiled extracts of plant tissues and of cyanide on the activity of succinoxidase from beef heart. Reaction medium contained 0.04 M. phosphate buffer (pH 7.0), 0.05 M. potassium succinate, and 0.3 ml. enzyme suspension. 0.001 M. HCN and/or 0.6 ml. boiled extracts were added as indicated. Final volume, 2.0 ml. Temperature 30°. Gas exchange measured for one hour.

Addition	Succinate oxidation	Inhibition	
		by extract	by extract plus HCN
	O ₂ μ l.	%	%
None	125		
+ HCN	0		100
+ <i>Lysichiton</i> spadix extract	20	80	
+ " + HCN	14		90
+ Tobacco leaf extract	53	60	
+ " + HCN	0		100
+ Beet leaf extract	56	60	
+ " + HCN	0		100

Table 5. Effects of ash from boiled extracts of plant tissues and of cyanide on the activity of succinoxidase from beef heart. Reaction medium contained 0.04 M. phosphate buffer (pH 7.0), 0.05 M. potassium succinate, and 0.3 ml. enzyme suspension. 0.001 M. HCN and/or ash from 0.6 ml. boiled plant extracts were added as indicated. Final volume, 2.0 ml. Temperature 30°. Gas exchange measured for one hour.

Addition	Succinate oxidation O ₂ μ l.	Inhibition	
		by ash	by ash plus HCN
None	131		
+HCN	0		100
+ <i>Lysichiton</i> spadix ash	90	30	
+ " +HCN	0		100
+Tobacco leaf ash	88	30	
+Beet leaf ash	76	40	

基質ははっきりしないが、少なくともコハク酸ではないらしい。

摘 要

ミズバショウ花穂から得られたミトコンドリアの自家呼吸は同じ組織の熱抽出液添加により抑制されるが、この抑制は青酸を添加することにより著しく

除去される。ミトコンドリアのコハク酸酸化能は非常に弱く、青酸によっても熱抽出液によっても抑制され、両者の間に拮抗は見られない。牛心筋から調製したコハク酸酸化酵素系も花穂の熱抽出液によって抑制されるが、この抑制作用は青酸阻害といくらか拮抗する傾向を示す。これらの結果から花穂の呼吸にみられる青酸促進の機作について考察した。

文 献

- 1) James, W. O., and Beevers, H., *New Phytol.* **49**: 353 (1950).
- 2) James, W. O., and Elliott, D. C., *Nature* **175**: 89 (1955).
- 3) Chance, B., and Hackett, D. P., *Plant Physiol.* **34**: 33 (1959).
- 4) Kuwayama, Y., and Usami, S., *J. Hokkaido Gakugei Univ. II B* **11**: 18 (1960).
- 5) Bonner, W. D., and Yocum, C. S., *Plant Physiol.* **31**: suppl. xli (1956).
- 6) Bendall, D. S., and Hill, R., *New Phytol.* **55**: 206 (1956).
- 7) Yocum, C. S., and Hackett, D. P., *Plant Physiol.* **32**: 186 (1957).
- 8) MacDonald, I. R., and DeKock, P. C., *Physiol. Plantarum* **11**: 464 (1958).
- 9) Hackett, D. P., and Simon, E. W., *Nature* **173**: 162 (1954).

Summary

The endogenous oxygen uptake of mitochondria from *Lysichiton* spadix was inhibited by boiled extract of the tissues and the inhibition was greatly removed by the addition of cyanide. Weak succinate oxidation of the mitochondria was inhibited by either cyanide or the boiled extract; both inhibitors scarcely competed with each other. This was also the case for succinoxidase from beef heart, excepting that a slight competition between these two inhibitors was observed. From these findings the mechanism of cyanide-stimulation of the spadix respiration was discussed.

日本植物学会第26回大会

プログラム

大会会長 小 倉 謙

学会会長 服 部 静 夫

会 期 10月13日(金)―10月15日(日)

会 場 東京大学理学部・医学部・薬学部

東京 1961

日 程

		会 場 9		10	11	12	13	14	15	16	17時	夜
第 1 日 13日 (金)	A	1 - 10 生 理・生 化				昼	11 - 24 生 理				微細構造談話会 分類学会 若者の集まり	
	B	1 - 7 生 理					8 - 19 生 理					
	C	1 - 7 遺 伝					8 - 20 生 態					
	D					食	1 - 12 細 胞					
	E	1 - 8 細 胞・形 態					9 - 22 分 類・形 態					
第 2 日 14日 (土)	A	25 - 34 生 理・生 化				昼 食・記 念 撮 影	総 会 (A 会 場)					藻類学会 生態の集まり
	B	20 - 29 生 理・生 化						シンポジウム 2				
	C	D13-D18 細 胞・形 態										
	D											
	E	23 - 32 分 類・形 態						シンポジウム 1				
第 3 日 15日 (日)	A	35 - 48 生 理				懇 親 会 (山 上 会 議 所)	シンポジウム 3				生理談話会	
	B	30 - 42 生 理・生 化・形 態					シンポジウム 4					
	C	21 - 30 生 態										
	D	19 - 31 細 胞										
	E	33 - 42 分 類・地 理										

特別講演 10月15日 18.00~19.00 (E会場)

Eric Hultén: Circumpolar Distribution of Plants (6×6 幻燈付)

(同氏は 13 日の分類学会にも出席されます)

- A: 薬学部記念講堂
- B: 医学部本館三階講堂
- C: 医学部一号館一階講堂
- D: 医学部本館一階講堂
- E: 理学部二号館四階講堂

A 会 場 [生 理・生 化]

9.00—10.15

- A 1 {藤 田 善 彦* 東 大・応微研 フィコビリ色素の暗生成過程における光化学反応活性の
服 部 明 彦 変化について
- A 2 {加 藤 栄* 東大・理・生化 葉緑体銅たんぱく Plastocyanin に関する諸光化学的反応
高 宮 篤 について
- A 3 {千 葉 保 胤* 九 大・理・生 葉緑体の酵素処理と、その光化学活性
岡 山 繁 樹
- A 4 渡 会 彰 彦 北 大・理・植 葉緑体の褪色と蛍光
- A 5 千 葉 保 胤 九 大・理・生 葉緑体中の蛍光物質について

10.15—10.30

総 合 討 論

10.30—11.45

- A 6 {菅 原 淳* 九 大・理・生 葉緑体への P^{82} のとりこみ IV. 光照射によるリピド分画
千 葉 保 胤 へのとりこみの増加
- A 7 宮 地 重 遠 東 大・応微研 ホウレンソウ緑葉中におけるポリリン酸
徳 川 生 研
- A 8 西 田 晃二郎 金沢大・理・植 光照射下に ^{14}C -glucose, 1,5- ^{14}C -citrate から放出される
 $^{14}CO_2$ について
- A 9 野 口 市 夫 東 大・理・植 葉緑体中に含まれるクエルセチン分解酵素について
- A 10 {藤 茂 宏* 岡山大・理・生 高等植物の光合成能の消長に関する研究 (第二報)
和 田 善 徳

11.45—12.00

総 合 討 論

B 会 場 [生 理]

10.00—11.00

- B 1 須 藤 俊 造 水 産 研 究 所 アサクサノリ類の生長、成熟などに対する日長効果
- B 2 杉 野 守 近 畿 大・農 アルビノコムギの日長反応
- B 3 江 刺 洋 司 東北大・理・生 シュウカイドウの短日反応に見られる $FR \rightleftharpoons Blue, Green$
および Red の可逆性について
- B 4 {滝 本 敦* 京大・農・応植 種々の波長光下で育成したアサガオの日長感受性
内 藤 佳 之

11.00—11.10

総 合 討 論

11.10—11.55

- B 5 {木 村 和 義* 京大・農・応植 低温によるアサガオの花芽形成
滝 本 敦 敦
- B 6 {丸 重 啓 二* 京大・農・応植 アサガオにおける花芽分化とたんぱく質分化
丸 重 靖 子
- B 7 寺 岡 宏 北 星 女 子 短 大 春化処理コムギ胚における窒素代謝

11.55—12.05

総 合 討 論

C 会 場 [遺 伝]

10.00—11.45

- C 1 {木 村 劫 二* 岡山大・理・生 ふたたびウシグソヒトヨの三核性子実体について
角 谷 啓 作
- C 2 竹 村 英 一 教育大・理・植 ヒガンバナ属の人工雑種 (第 5 報)
- C 3 向 川 信 一 北 大・理・植 トウモロコシの混合受粉

C 4	武 丸 恒 雄	岡山大・理・生	帽菌類におけるヘテロカリオン形成
C 5	坪 由 宏	神戸大・理・生	ストレプトマイシンにより誘発されるクラミドモナスの葉緑体変異について
C 6	{ 奥 田 正 男* 柳 島 彦 夫 高 田 英 夫	大阪市大・理・植	合成界面活性剤による酵母の呼吸欠損変異の誘起
C 7	竹 中 要	国立遺伝研	染井吉野の起源の決定と原産地の推定
11.45—12.00			総 合 討 論

E 会 場 (細胞・形態)

9.00—10.00			
E 1	左 貝 アイ子	奈良女子大・理・植	植物細胞のオスミウム固定についての電子顕微鏡的研究
E 2	{ 上 野 実 朗* 北 口 貞 夫	大阪市大・理・植	電子顕微鏡によるヒツジグサ科花粉膜の微細構造
E 3	村 上 悟	東大・理・生化	オオムギの根の色素体の prolamellar body(ラメラ形成体)
E 4	村 上 悟	東大・理・生化	葉緑体の grana lamellae の微細構造
10.00—10.15			総 合 討 論
10.15—11.15			
E 5	{ 遠 山 益* 植 田 利 喜 造	教育大・理・植	キャベツ葉の色素体の発達と構造に関する電子顕微鏡的研究
E 6	{ 大 隅 正 子* 湯 浅 明	日本女子大・生 東大・教養・生	電子顕微鏡による有色体の研究
E 7	川 松 重 信	愛知学芸大	アカウキクキの根のプラスチックについて
E 8	湯 浅 明	東大・教養・生	シダ植物の細胞学的研究 XXXV. 色素体の自律性
11.15—11.30			総 合 討 論

A 会 場 (生 理)

13.00—14.45			
A11	上 坪 英 治	阪 大・理・生	周回型原形質流動の向きについて
A12	阿 部 重 美	阪 大・理・生	原形質運動と -SH
A13	高 沖 武	広島大・理・植	根の吸水機構に関する研究 (I)
A14	{ 神 谷 宣 郎* 田 沢 立 堯 仁 子	阪 大・理・生	フラズモ節間細胞の透過性
A15	{ 田 沢 立 堯 仁 子* 足 立 堯 仁 子	阪 大・理・生	細胞横断渗透法によるフラズモ細胞のアルコールにたいする透過性の決定
A16	{ 永 井 玲 子* 田 沢 仁	阪 大・理・生	ヒメフラズモに見られる光電反応とイオン吸収
A17	{ 玉 井 直 人* 西 田 見 二郎	金沢大・理・植	光合成産物の根への転流におよぼす光の影響
14.45—15.00			総 合 討 論
15.00—16.45			
A18	賀 来 章 輔	下関商業高校 福岡学芸大・生	植物組織の凍結曲線の分析 (4)
A19	衣 川 堅 二郎	京大・農・応植	キヌガサタケの生長について II. 特に pH との関係
A20	今 井 百里江子	お茶の水大・理・植	絶対好稠性を示す一新糸状菌について (第2報)

A21	松 下 亀 久	九 大・理・生	TMV の増殖に関する研究 そのⅢ
A22	相 馬 悌 介	新潟大・教 育	気孔細胞などの顕微解剖手術法
A23	藤 野 正 義	長崎大・学芸・生	気孔の開閉と ATP, ATP-ase
A24	巖 佐 耕 三	阪大・教養・生	セン類 (<i>Funaria hygrometrica</i>) の原系体培養 (予報)
16.45—17.00			総 合 討 論

B 会 場 (生 理)

13.00—14.15

B 8	{ 内 田 譲 昭*	京 大・理・植	酵母の突然変異に対する銅の効果
B 9	荒 勝 豊	甲南大・理・生	含銅培地上での酵母の変異誘起における初期培養条件の意義
B10	{ 瀬 野 悍 二*	京 大・理・植	酵母の銅耐性と細胞の銅の取込み
B11	{ 庄 司 善 哉*	東北大・農・農	アデニン要求性酵母の増殖異常
B12	{ 三 戸 信 人*	千葉大・腐敗研	酵母細胞の密集状態における生理的变化
14.15—14.30			総 合 討 論

14.30—15.15

B13	鳥 山 英 雄	東京女子大・文 理・生	オジギソウの細胞の生理学的研究 (第15報) 一葉柄の節部の基本構造について一
B14	須 田 省 三	神戸大・理・生	オジギソウの刺激物質
B15	柴 岡 孝 雄	東北大・理・生	オジギソウの興奮性細胞における伝導
15.15—15.30			総 合 討 論

15.30—16.30

B16	照 本 勲	北 大・低温研	マリモの凍害と多価アルコール
B17	{ 渡 辺 篤* 清 原 千 里	東 大・応微研	地衣、苔およびソテツと共生するラン藻について
B18	林 克 己	広島大・理・植	トウキビとコムギの芽ばえの生長におよぼすビタミン類の影響
B19	堀 武 義	岐阜大・学 芸	マツバボタンの花の開閉に及ぼす光、熱の効果
16.30—16.45			総 合 討 論

C 会 場 (生 態)

13.00—14.30

C 8	中 西 哲	神戸大・教 育	着生群落ナガスジトゴケ群集について
C 9	{ 堀 川 芳 雄* 岡 本 香	広島大・理・植	広島県地質とスゲ属植物について
C10	小 村 精	九 大・理・生	福岡市周辺山地の森林植生
C11	{ 田 川 日出夫* 宮 田 逸 夫	九 大・理・生	桜島の植生構造 V. 東部低木林の組成と成因
C12	北 川 昌 典	滋賀・土山小学校	鈴鹿山系におけるマツ型森林の発達 (第一報)
C13	南 川 幸	三重・菟野高校	鈴鹿山脈ツブラジイ林の天然更新に関する考察

14.30—14.45

総 合 討 論

14.45—15.45

- C14 菅 沼 孝 之 奈良女子大・理 植 福島県檜枝岐村の花沼湿原の植物群落 (予報)
- C15 吉 岡 邦 二 東北大・理・生 森林の退化によるミズゴケ湿原形成
- C16 { 辻 井 達 一* 北 大・農 泥炭地の植物群落遷移について
小 塚 芳 道
- C17 { 沼 田 登 真* 千葉大・文理・ 遷移からみた埋土種子集団の解析
小 村 志 子
大 木 一 方
林 一 方

15.45—16.00

総 合 討 論

16.00—16.45

- C18 高 橋 基 生 東 大・理・植 植生の特異分布ならびに生育に対する植物生態学的研究
I. 温度変異に基づく植生異常(第2報). 神津島における
環境要因ならびに植生の特異性とその御蔵島との比較
- C19 高 橋 基 生 東 大・理・植 同上 II. 偶然要因に基づく植生異常(第1報). 伊豆諸島
における植生分布を支配する環境要因と偶然性について
- C20 高 橋 基 生 東 大・理・植 同上 III. 水分経済または根系呼吸の破綻,あるいは養料
失調に基づく植生異常(第1報). 奥羽地方日本海岸寄り諸
高山における亜高山帯針葉樹欠除に対する生態学的見解

16.45—17.00

総 合 討 論

D 会 場 (細 胞)

13.00—14.00

- D1 辰 野 誠 次 広島大・理・植 *Selaginella* 属の細胞学的研究 II
- D2 藤 原 悠紀雄 神戸大・御影分 校・生 *Aster* 属の核型分析 (第7報)
- D3 竹 本 貞一郎 岡山大・教育・ 生 ニガナ群の細胞学的研究 (続報)
- D4 神 野 太 郎 愛媛大・教育・ 生 *Polygonatum* (ナルコユリ属) 数種の染色体

14.00—14.15

総 合 討 論

14.15—15.15

- D5 下斗米 直 昌 広島大・理・植 染色体数の異なる種の間自然雑種の細胞学的研究
- D6 茅 野 博 九 大・理・生 ホウチャクソウにおける染色体不対合と非減数花粉粒の形成
- D7 { 松 浦 一 樹* 北 大・理・植 NaCl, KCl, CaCl₂ 処理によって誘発された減数分裂の異常
岩 淵 雅
- D8 { 松 浦 一 樹* 北 大・理・植 還元分裂における半染色体体組換え像の出現について
谷 茂 雅
金 淵 沢 行 樹
岩 金 甫

15.15—15.30

総 合 討 論

15.30—16.30

- D9 大 野 林二郎 北 大・理・植 センブリ (*Swertia japonica*) 抽出液による花粉母細胞分
裂の異常
- D10 劉 逸 民 北 大・理・植 オオバナノエンレイソウの花粉母細胞分裂における高温処
理の影響

D11	加藤 一男	京大・理・植	中間期における染色体の行動
D12	植田 勝己	奈良女子大・理・植	8ミリ映画によるムラサキツユクサの細胞の有糸分裂の観察
16.30—16.45			総 合 討 論

E 会 場 (分 類・形 態)

13.00—14.45

E 9	石川 元助		トリカブト属植物とその毒矢文化圏
E 10	増田 染一郎	三生製薬	湿室培養によって分離した <i>Myxobacteria</i> の新種について
E 11	棒 啓介	酸酵研究所	石狩川河水の汚染糸状菌について
E 12	{ 信島 隆善 治* 川 戸 峯 子	大阪芸大・平野分校	Whirl を形成する放線菌の一新種 <i>Streptomyces griseoverticillatus</i> について
E 13	{ 川 戸 峯 子* 信 夫 隆 治	大阪芸大・平野分校	放線菌の窒素源利用について II. 炭素源に glucose を用いた場合の NO_2^- と NO_3^- について
E 14	米山 穰	広島大・教養	A new heterothallic yeast, <i>Endomycopsis scolyti</i>
E 15	曾根田 正己	長尾研究所	海産魚類腸管中の酵母について
14.45—15.00			総 合 討 論

15.00—16.45

E 16	小林 艶子	横浜市大・文理・生	羽状ケイ藻 <i>Ceratoneis arcus</i> Kütz の変異
E 17	{ 熊野 茂* 瀬戸 良三 広瀬 弘幸	神戸大・理・生 神戸女子学院・高等部 神戸大・理・生	カワモズク属の一種 <i>Batrachospermum ectocarpum</i> Sirod. の種内の変異および他種との関係について
E 18	{ 瀬戸 良三* 熊野 茂 広瀬 弘幸	神戸女子学院・高等部 神戸大・理・生 神戸大・理・生	カワモズク科数種の <i>Chantransia</i> stage の比較
E 19	笠原 和男	北大・理・海藻研	コンブ科の粘液腔道について
E 20	{ 猪野 俊平* 西林 長朗	岡山大学・理・植	ツルアラメの遊走子囊発生と遊走子形成
E 21	広瀬 弘幸	神戸大・理・生	淡水産シオグサ科 <i>Basidiocladia</i> 属の一新種について
E 22	千原 光雄	教育大・臨海実験所	緑藻プラシノクラズ・アスクスの生活史とその類縁についての一考察
16.45—17.00			総 合 討 論

A 会 場 (生 理・生 化)

9.00—10.45

A 25	{ 武林 幸作* 林 孝三	教育大・理・植	パンジーの紫色色素について
A 26	柴田 万年	富山大・文理・生	チューリップの一品種 Charles Needham の花のアントシアン
A 27	{ 石倉 茂行* 林 孝三	教育大・理・植	ダイコンの赤色根皮の anthocyanin について
A 28	{ 菊地 正彦* 中 原 正 城	教育大・理・植	<i>Penicillium islandicum</i> Sopp. の紫外線照射株における色素産生の消長

- A29 三 井 清 司 教育大・理・植 生 総合 討論
10.15—10.30
10.30—11.45
- A30 村 上 進 埼玉大・文理・生 リボングラスのポリフルクトサンについて
- A31 { 西 沢 一 俊* 教育大・理・植 紅藻でんぶんの生化学的研究
尾 崎 一 郎
- A32 { 入 来 義 彦* 信州大・教育・ 緑藻細胞膜間粘質物の生化学的研究(II). 緑藻 *Collinsiella*
三 輪 知 雄 教育大・理・植 の細胞膜間粘質物について
- A33 { 武 田 宏* 教育大・理・植 カワノリの細胞膜間質
三 輪 知 雄
- A34 森 祐 二 奈良医大・細菌 細菌細胞壁溶解酵素と protoplast
11.45—12.00 総合 討論

B 会 場 (生 理・生 化)

- 9.00—10.00
- B20 { 鈴 木 昇* 愛知女子大・生 生 *Azotobacter* のピリジンスクレオチド
奥 木 聡 旺 名大・教養・生
鈴 木 旺 愛知女子大・生
- B21 浅 田 弘 治 広島大・理・植 食塩高張培地における *Bacillus subtilis* の RNA
- B22 中 村 運 甲南大・理・生 酵母の重金属 Cross Resistance におけるリボ核酸代謝の意義
- B23 { 木 村 孝 一 山 口 医 大・生 高等植物葉緑体の RNA 抽出法に関する考察
新 田 毅* 東 大・理・植
10.00—10.15 総合 討論
- 10.15—11.45
- B24 沢 井 輝 男 愛知学芸大名古 Candida amylase のでんぶん質よりの glucose 転移につ
屋分校・生 いて
- B25 加 藤 勇 夫 広島大・教養・ 陰花植物ならびに二, 三の顕花植物における phosphory-
生 lase および amylase 作用
- B26 { 菊 池 忠 寿* 京 大・理・植 酵母銅耐性株のいおう代謝
芦 田 譲 治
- B27 内 貴 信 夫 岐阜大・学芸・ 酵母のメチオニン要求株を用いたいおう代謝の研究
生
- B28 { 服 部 明 彦* 東 大・応微研 ラン藻 *Anabaena cylindrica* の硝酸還元系について
渡 辺 篤 篤
- B29 福 田 育 二 郎 理科大・理・生 耐熱性ラン藻 *Cyanidium* の窒素代謝 (第二報)
11.45—12.00 総合 討論

D 会 場 (細 胞・形 態)

- 9.00—9.45
- D13 { 太 田 次 郎* お茶の水大・理 変形体の振とう培養について
岡 田 順 子 ・植
- D14 { 黒 田 清 子 阪 大・理・生 細胞外に遊離した原形質滴の行動
神 谷 宣 郎*
- D15 加 藤 幸 雄 名 大・理・生 シダの初期前葉体における細胞の単離
9.45—10.00 総合 討論

10.00—10.45

- D16 {藪野恭三* 阪大・理・生 遠心処理したヒメフラスモ節間細胞について
清水 晃
- D17 中沢信午 山形大・文理・生 有極性原形質分離
- D18 八戸正夫 熊本大・教育・生 ユキノシタの葉の表皮細胞の原形質分離時の季節的变化と日変化について
- 10.45—11.00 総 合 討 論

E 会 場 (分 類・形 態)

9.00—10.00

- E23 加崎英男 都立大・理・生 日本新産属 *Lamprothamnium* (Characeae) について
- E24 岩崎尙彦 都立大・理・生 ジャクモ科植物の生長点の分化と器管形成 VI. *Toly-pella gracilis*
- E25 {堀川芳雄 広島大・理・植 *Brotherella recurvans* (Mich.) Fleisch, ミヤマカガミゴケ (セン類) について
関 太郎*
- E26 百瀬静男 文 部 省 シダにおける無配生殖と種の分化
- 10.00—10.15 総 合 討 論

10.15—11.45

- E27 増淵法之 北大・理・植 コムギ分枝穂の形態学的研究
- E28 高木虎雄 京都・園部高校 ササ属の花の分類学的知見
- E29 鈴木貞雄 宇都宮中央女子高校 関東・東北地方産ササ属, チシマザサ節の分類
- E30 小宮定志 日本歯大・生 南アフリカ産 *Roridula* の解剖学的知見
- E31 桃谷好美 京大・理・植 たんぱく質から見たカエデ属の類縁 (第二報)
- E32 豊国秀夫 北大・理・植 リンドウ属の分割について
- 11.45—12.00 総 合 討 論

A 会 場 (生 理)

9.00—10.45

- A35 黒田清子 阪大・理・生 正常および腫瘍組織による組織発生の誘導現象について
- A36 三木寿子 京大・理・植 *Primula obconica* における花粉管の負の屈性
- A37 田中清 福島大・学芸・生 アカマツ花粉に含まれる酸性生長抑制物質の発芽に伴う量的変化
- A38 柴岡弘郎 東大・植物園 カフェー酸の生長阻害作用
- A39 小林万寿男 東京学芸大・生 茎における不定根発根の抑制
- A40 末本雛子 京大・農・遺伝 一粒小麦の左右性の方向決定に対するインドール酢酸の効果
- A41 柳島直彦 大阪市大・理・生 呼吸欠損酵母にみられるオーキシンの細胞伸長促進作用
- 10.45—11.00 総 合 討 論

11.00—12.45

- A42 沢田義康 北海道学芸大・旭川分校・生 雌ずいおよび雄ずいに含まれるオーキシン含量について
- A43 {和田俊司* 東北大・理・生 イネの子葉鞘中のインドール酢酸酸化酵素阻害物質 (統報)
長尾 昌之

A44	{中村輝子 石井慎義 八巻敏雄	東大・教養・生	アベナ子葉鞘の細胞内のオーキシン分布
A45	{岡上伸雄 江刺洋司 長尾昌之	東北大・理・生	ジベレリンによるシュウカイドウの無性芽形成の抑制と促進作用について
A46	加藤次郎	大阪府大・教養・生	タケノコのジベレリン様物質について
A47	村上浩	農 技 研	ジベレリンの植物体内における変化
A48	{高橋憲子 師尾武子 八巻敏雄	日本女子大・生 日本女子大・生 東大・教養・生	タバコ種子の暗発芽に対する数種の有機酸の作用と pH との関係
12.45—13.00			総 合 討 論

B 会 場 (生理・生化・形態)

9.00—10.30			
B30	山本昌木	島根農大	<i>Phytophthora infestans</i> (Mont.) De Bary 菌胞子発芽の二型について
B31	{今堀宏三 巖佐耕三	阪大・教養・生	ジャクモの発芽と生長の環境コントロール
B32	井上浩	教育大・理・植	ゼニゴケ胞子の発芽と光の照射時間との関係
B33	河原晨	大阪市大・理・生	ヒシモドキの発芽について
B34	山田見弘	東大・教養・生	トウモロコシ発芽時にみられる CoA 量と ATP 量
B35	宮本義男	愛媛大・文理・生	微生物によるパラフィンおよびろうの分解 (続報)
10.30—10.45			総 合 討 論

10.45—12.00

B36	{小野田哲夫 宇佐美正一郎	北 大・理・植	<i>Staphylococcus aureus</i> 青酸耐性菌の呼吸について
B37	{村山徹郎 芦田譲治	愛媛大・文理・生 京 大・理・植	銅耐性酵母の TCA サイクルについて
B38	{田中滋郎 宇佐美正一郎	北 大・理・植	サトウダイコンの根の呼吸系について
B39	{熊谷孝美 宇佐美正一郎	北 大・理・植	トマトとイヌホオズキのつぎ木による周縁キメラ雑種の呼吸について
B40	佐々木喜美子	北 大・理・植	ペゴニアの葉の呼吸酵素について
12.00—12.15			総 合 討 論

12.15—12.45

B41	{辻田英夫 濱田秀男	兵庫農大・生	イネ芽ばえのエネルギー代謝形式変動の乾燥重量への反映
B42	{駒嶺穆夫 服部静夫	東 大・理・植	ハッショウマメにおけるチロシンの代謝
12.45—13.00			総 合 討 論

C 会 場 (生 態)

9.00—10.00

C21	矢野 悟 道	広島大・理・生	草原における植物地下器官の生態学的考察, 特に放牧地について
C22	{ 宮脇 昭* 大 場 達 之	横浜国大・学芸・生	奄美群島の海浜植生
C23	倉内 一二	豊橋東高校	伊勢湾台風の害と回復状況—塩風害と海岸林 III
C24	{ 延原 肇* 小 滝 一 夫	千葉・習志野高校	塩沼群落の問題点
10.00—10.15			総 合 討 論

10.15—11.00

C25	柳 沢 新 一	埼玉県蚕業試	発育の法則について, 付・生態学的限界に対する考察
C26	{ 岩城 英 夫* 翠 川 文 次 郎	東大・理・植 都立大・理・生	霧ヶ峯草原群落の現存量と生産構造に対する採草の影響
C27	大島 康 行	都立大・理・生	数種のササの耐陰性
11.00—11.15			総 合 討 論

11.15—12.00

C28	樫村 利 道	福島大・学 芸	ブナ林植物の葉の日補償点
C29	野本 宜 夫	茨城大・文理・生	ブナ林の生産構造について
C30	{ 小林 弘* 市 村 俊 英	教育大・理・植	河川底生藻類の光合成について
12.00—12.15			総 合 討 論

D 会 場 (細 胞)

9.00—10.30

D19	{ 高田 英 夫* 山 本 武 夫	大阪市大・理・生	高張ストロンチウムの溶液中の酵母原形質片の形成とその再生
D20	{ 山本 武* 高田 英 夫	大阪市大・理・生	ストロンチウム高張環境における酵母原形質の顆粒化とその可逆性
D21	米田 芳 秋	国立遺伝研	酵母核の構造について
D22	中村 威	京都学芸大・生	紡錘体および隔膜形成体の構造について
D23	松浦 一	北大・理・植	自然における染色体切断の一例
D24	{ 松浦 一* 武 久 慎	北大・理・植	<i>Trillium</i> の meiotic metaphase I の染色体構造におよぼす EDTA の効果
10.30—10.45			総 合 討 論

10.45—11.30

D25	馬場 三 吾	京大・理・植	カルス形成過程における mitotic index の変化と二, 三の酵素活性の変化
D26	{ 松浦 一* 佐谷 茂 雅 岩 淵 樹	北大・理・植	X線誘起染色体異常におよぼす ATP と DNA の効果
D27	{ 松浦 一* 佐谷 茂 雅 岩 淵 樹	北大・理・植	X線誘起染色体異常におよぼす Mitomycin C の効果
11.30—11.45			総 合 討 論

11.45—12.45

D28	{ 山崎 典 子* 水 野 忠 欽	慶応大・生	コアツモリ染色体における加水分解の時間とフォイルゲン反応との関係について
-----	----------------------	-------	--------------------------------------

D29	山岸英夫	京大・理・植	<i>Hydrodictyon reticulatum</i> の生長ともなう蛍光染色性の変化
D30	新家浪雄	京大・理・植	^3H -thymidine の細胞核への取り入れ
D31	吉田吉男	新潟大・理・生	葉緑体の働きにおよぼす細胞核の相関性
12.45—13.00		総	合 討 論

E 会 場 (分 類・地 理)

9.00—10.15

E33	丸山 晃	国立自然教育園	Mauritius 島のラン藻類
E34	{福 島 博* 入 山 陸 子	横浜市大・文理・生	南極大陸問題岩露岩帯のケイ藻
E35	{福 島 博* 星 野 郁 子	横浜市大・文理・生	南極大陸新南露岩帯のケイ藻
E36	大野正夫	横浜市大・文理・生	北海道知床半島ポロモイ台地のケイ藻類
E37	奥野春雄	京都工芸繊維大	北海道における海成ケイ藻土
10.15—10.30		総	合 討 論

10.30—11.00

E38	野田光蔵	新潟大・理・生	日本海佐渡島に発生する緑藻ヒトエグサ (<i>Monostroma</i>) について
E39	越智春美	鳥取大・学芸・生	日本およびその近接地域産タマゴケ科セン類の分布について
11.00—11.15		総	合 討 論

11.15—12.00

E40	三木 茂	大阪市大・理・生	遺体フロラからみた邦産水生植物
E41	粉川昭平	大阪市大・理・生	ミツガシワの過去と現在の分布
E42	里見信生	金沢大・理・生	塩素酸カリの抗毒性による本州内外帯植物の判別
12.00—12.15		総	合 討 論

シンポジウム

10月14日 14.30~17.00

話題 1 変異性の問題 (E 会場)

- | | | |
|-----------|-----------|-------------------|
| (1) 西岡 泰三 | 都立大・理・生 | ニガナの種内変異の細胞遺伝学的研究 |
| (2) 山崎 敬 | 東大・理・植 | ササ類の分化と環境 |
| (3) 永井 進 | 奈良女子大・理・生 | 酵母菌類の形質的安定性と不安定性 |

話題 2 細胞の構造と機能 (B 会場)

- | | | |
|-----------|--------|------------------------|
| (1) 岡本 尚 | 名大・理・生 | 細胞膜の構造と機能 |
| (2) 茅野 春雄 | 東大・理・動 | カイク卵の休眠中における糖代謝調整とその機構 |

10月15日 14.30~17.00

話題 3 Species population の問題 (A 会場)

- | | | |
|-----------|------------|--------------------------------------|
| (1) 石塚 和雄 | 岩手大・一般教育・生 | 種集団の複合としての植物共同体—八甲田山高田谷地の湿原植生を中心として— |
| (2) 黒岩 澄雄 | 東大・理・植 | 種集団の成立と競争について |
| (3) 福田 一郎 | 東京女子大・文理・生 | Population の変遷 |

話題 4 細胞と組織の分化 (B 会場)

- | | | |
|-----------|---------|----------------------|
| (1) 林 俊郎 | 東大・教養・生 | 高等植物における細胞培養の発展とその応用 |
| (2) 佐藤 七郎 | 東大・理・植 | 植物組織分化の研究の方法について |

大会関係の会議・集会

10月12日(木)

- | | | | |
|------------------|--------------|-------------|----------|
| 植物学会評議員会 | 学士会館(本郷) | 17.00—21.00 | |
| 植物学研究連絡委員会 | 学士会館(本郷) | 13.30—16.30 | |
| シダ学会 | 東大・植物学教室・講義室 | 9.00から | |
| 日本菌学会 | 斯文会 | 15.00から | 会費 100 円 |
| 酵母細胞研究会 | 養賢堂会館 | 13.00から | |
| (ひきつづき懇親会があります。) | | 18.00から | |

10月13日(金)

- | | | | |
|---------|----------|--------------|----------|
| 微細構造談話会 | 東大出版会集会室 | 18.30から(夕食後) | |
| 分類学会 | 学士会館(本郷) | 17.30—20.30 | 会費 500 円 |
| 若者の集まり | 東大・山上会議所 | 17.00—20.00 | |

10月14日(土)

- | | | | |
|--------|----------------------|------------------|--|
| 藻類学会 | 時間、会場未定、大会当日お知らせします。 | | |
| 生態の集まり | 東大・山上会議所 | 18.00—20.00(夕食後) | |

10月15日(日)

- | | | | |
|-------|-----------------|--|--|
| 生理談話会 | 詳細は大会当日お知らせします。 | | |
|-------|-----------------|--|--|

10月16日(月)

見学 11 時ごろ、水戸駅に集合。上野帰着は夜 8 時ごろの予定。列車時刻の改正が予定されていますので、細かいことは会場でお知らせします。

会場案内図



Fruit Body Formation of Red Bread Mold *Neurospora crassa* IV. Effect of Ammonium and Nitrate Ion in the Medium on Size of the Perithecium

by Taro ITO*

Received September 3, 1961

A study on the mechanism of the production of the perithecium in the fertilization stage of the sexual reproduction process, including protoperithecial formation, ascospore production and fruit body formation, should be generally completed after information upon their genetical and physiological natures has been obtained. In physiological study on the effect of concentration of KNO_3 on the formation of the female sexual organ and fruit body, Hirsch¹⁾ has suggested that the existence of the nitrate ion had no direct relation to the fertilization and the fruit body development, but showed an effective influence on the differentiation of the female sexual organ. Further he showed that the exhaustion of the nitrate ion in the culture medium was not necessary for the formation of a female sexual organ, but for the formation of perithecia.

In the sexual reproduction, the process of the formation of the fruit body following sexual organ formation seems to be one of morphogenesis. Therefore, in connection with the investigation on the morphogenesis of this fungus, an analysis of the external and internal factors which affect the reproductive growth phase, seems to be a subject of considerable importance. In previous papers^{2,3)}, the author has shown that the perithecia were produced in the culture medium containing both ammonium and nitrate salts in a particular ratio, and that perithecia without ascospore were formed on the single strain mycelium in the culture filtrate which was obtained from the culture medium containing these two nitrogen salts at the beginning of the fertilization. These investigations involve the examination on the factors controlling the cell formation (heterogenous development), in association with the expression of sexuality in morphogenesis. For the further understanding of the fruit body formation, it may be necessary to analyse the factors affecting perithecial enlargement (homogenous development). The present report deals mainly with the effects of concentration ratio of nitrate to ammonium ion on the development of perithecial size.

Materials and Methods

The strains used in this experiment are designated as 4A and 8a. They are macroconidial wild type strains which form fruit bodies, after 10 to 14 days, on synthetic media containing inorganic salts, inorganic nitrogen, suitable organic carbon and the vitamin biotin. The minimum medium used for this experiment is the crossing medium designated by Westergaard and Mitchell⁴⁾. The four kinds of minimal media were prepared for the test of perithecial formation. The medium used for the study on the effects of ammonium and nitrate ion in the culture mixture was the one supplemented with ammonium tartrate of 10 different concentrations from 0.5×10^{-3} M. to 0.5×10^{-2} M. and potassium nitrate (10^{-2} M.). In the other culture series, KNO_3

* Laboratory of Botany, Dairy Section, Obihiro Zootechnical College, Obihiro, Japan.

solutions were prepared at 10 different final concentrations from 10^{-3} M. to 10^{-2} M. with ammonium tartrate (10^{-2} M.). The effect of a sole nitrogen source was similarly tested by the independent application of one of these nitrogen sources in the concentration range from 10^{-3} M. to 2×10^{-2} M. The quantitative analysis for the residual ammonium- and nitrate-ion was carried out spectrophotometrically, and the hydrogen ion concentration in the liquid medium was also measured.

Experimental Results

In the first experiment, the influences of both nitrate and ammonium salts in the cross medium on protoperithecial and perithecial development were tested by measuring the diameter of perithecia formed on the slant medium in the test tube. The effect of CF on the formation of the protoperithecium and the perithecium was tested by the use of the crossing medium of Westergaard and Mitchell⁴). +CF was obtained from the liquid synthetic media, in which a single culture of A (+) type strain hyphae was made, and similarly -CF was prepared from the culture solution of a (-) type strain. Ten of both the protoperithecia and perithecia formed by 10 to 14 days incubation at 25° were picked up at random and their diameters were measured with the micrometer under microscope. The results are shown in Table 1-(I) & (II).

As seen in Table 1-(I), remarkable stimulation of the growth of perithecia was observed when both the ions were supplied at the ratios of 10:10, 5:10, 3:10 and 0:10. The effect of +CF upon the growth of perithecia was striking at the ionic ratios of 2:10 and 0:10, and that of -CF was at 10:10 and 7:10. Significant negative correlation was recognized between the concentration of ammonium ion and size of the perithecium. The correlation coefficient between the size of perithecia and the amount of ammonium or nitrate ion was higher in the ammonium minimum medium rather than in the nitrate minimum medium. It was also found that the perithecial growth

Table 1-(I). Size of perithecia formed on the culture media supplemented with the various amount of both ammonium and nitrate.

Conc. grade	0	1	2	3	4	5	6	7	8	9	10
Conc. ratio [NH ₄ ⁺]:[NO ₃ ⁻]	0:10	1:10	2:10	3:10	4:10	5:10	6:10	7:10	8:10	9:10	10:10
Range in size (μ)	583— 699.6	—	408.1— 583	466.6— 699.6	408.1— 524.7	408.1— 583	349.8— 583	349.8— 466.6	408.1— 583	349.8— 466.4	408.1— 583
Average size (μ)	641.3 ±18.5		489.6 ±19	606.2 ±24	478.0 ±4.6	512.8 ±18	442.6 ±19	419.4 ±6.5	478.0 ±19	396.2 ±19	500 ±28

Cor. co. (1)*: -0.8. Cor. co. (2)*: -0.5.

Conc. grade	11	12	13	14	15	16	17	18	19	20	21
Conc. ratio [NH ₄ ⁺]:[NO ₃ ⁻]	10:0	10:1	10:2	10:3	10:4	10:5	10:6	10:7	10:8	10:9	10:10
Range in size (μ)	—	—	—	349.8— 699.6	408.1— 583	408.1— 641.3	560.2— 594.6	349.8— 524.7	466.4— 699.6	408.1— 641.3	466.4— 583
Average size (μ)				489.6 ±30	510.2 ±21	501.2 ±21	583.0 ±0	478.0 ±21	537.6 ±25	501.2 ±30	513.1 ±21

Cor. co. (1)*: -0.01. Cor. co. (2)*: 0.2.

Table 1-(II). Size of perithecia formed on the crossing medium supplemented with the CFs from the culture of either A (+) or a (-)

+CF	Conc. grade	0	1	2	3	4	5	6	7	8	9	10
	Range in size (μ)	466.4—641.3	233.2—524.7	466.4—699.6	256.4—583	466.4—699.6	466.4—699.6	408.1—641.3	466.4—699.6	349.8—583	408.1—583	408.1—583
	Average size (μ)	559.2 ± 16.2	384.6 ± 25.5	570.8 ± 23.2	489.6 ± 16.2	537.6 ± 25.5	536.0 ± 23.2	537.6 ± 23.2	489.6 ± 23.2	489.6 ± 23.2	489.6 ± 16	478.0 ± 23.2
Cor. co. (1)*:—0.1.						Cor. co. (2)*:0.04.						
+CF	Conc. grade	11	12	13	14	15	16	17	18	19	20	21
	Range in size (μ)	174.9—233.2	291.5—524.7	233.2—408.1	233.2—466.4	—	174.9—349.8	326.0—466.4	349.8—641.3	349.8—524.7	233.2—466.4	—
	Average size (μ)	209.4 ± 2.5	361.4 ± 25	302.8 ± 5.8	326.0 ± 23	—	279.6 ± 23	421.0 ± 23	489.6 ± 30	421.0 ± 5.8	326.0 ± 28	—
Cor. co. (1)*:0.1.						Cor. co. (2)*:0.2.						
-CF	Conc. grade	0	1	2	3	4	5	6	7	8	9	10
	Range in size (μ)	349.8—583	—	291.5—466.4	349.8—583	349.8—524.7	349.8—524.7	408.1—583	524.7—757.9	291.5—583	349.8—524.7	408.1—583
	Average size (μ)	454.2 ± 27.8	—	333.0 ± 20.8	454.2 ± 25.5	442.6 ± 16.2	442.6 ± 16.2	501.2 ± 18.7	583 ± 18.7	419.4 ± 30.3	442.6 ± 16.2	489.6 ± 18.7
Cor. co. (1)*:0.3.						Cor. co. (2)*:0.6.						
-CF	Conc. grade	11	12	13	14	15	16	17	18	19	20	21
	Range in size (μ)	291.5—524.7	233.2—583	233.2—349.8	233.2—466.4	233.2—583	349.8—524.7	233.2—466.4	233.2—466.4	174.9—466.4	291.5—349.8	233.2—583
	Average size (μ)	419.4 ± 18	361.4 ± 30	291.5 ± 14	326.0 ± 26	421.0 ± 31	442.6 ± 17	373.0 ± 34	373.0 ± 29	337.6 ± 35	304.4 ± 15	361.4 ± 30
Cor. co. (1)*:0.05.						Cor. co. (2)*:0.1.						
Without any CFs**		Range in size (μ)	421.0—583									
		Average size (μ)	478.0 ± 14									

* The correlation coefficient between size and ammonium (1) or nitrate (2).

** The crossing medium without supplementation of the CFs.

decreased more strikingly with an increase in the amount of ammonium ion added than with that of nitrate ion. The positive correlation between the amount of ammonium ion and the size of perithecia formed was revealed by the application of -CF. In order to ascertain the difference of the effect of varied ionic ratios ($[\text{NH}_4^+]:[\text{NO}_3^-]=10^{-3}\sim 9\times 10^{-3}:10^{-2}$ or $10^{-2}:10^{-3}\sim 9\times 10^{-3}$) between the two lots, the analysis of variance of the means was calculated. No highly significant difference was observed between the means of the two lots ($P>0.05$).

The second experiment was carried out to see the effect of ammonium and nitrate minimum medium on the growth of perithecia and also to confirm the influence

of the high concentration of the sole nitrogen source of either ammonium or nitrate. The concentration range of the synthetic media was made up between 10^{-3} M. and 2×10^{-2} M. The results are shown in Table 2.

Table 2. Size of perithecia formed on the culture media supplemented with the sole nitrogen ion of either ammonium or nitrate.

Conc. grade	0	1	2	3	4	5	6	7	8	9	10
Conc. of $(\text{NH}_4)_2\text{C}_4\text{H}_4\text{O}_6$	0	$10^{-3} \times 0.5$	$\times 1$	$\times 1.5$	$\times 2$	$\times 2.5$	$\times 3$	$\times 3.5$	$\times 4$	$\times 4.5$	$\times 5$
Range in size (μ)	466.4–583	357.0–571.2	357.0–571.2	428.4–571.2	428.4–714	357.0–642.2	499.4–856.8	428.4–571.2	428.4–714	357.0–571.2	—
Average size (μ)	542.0 ± 28	485.2 ± 23	485.2 ± 23	485.2 ± 29	613.8 ± 30	499.4 ± 33	571.2 ± 39	499.4 ± 21.3	571.2 ± 25	527.8 ± 29	—

Cor. co. (1): 0.4.

Conc. grade	11	12	13	14	15	16	17	18	19	20
Conc. of $(\text{NH}_4)_2\text{C}_4\text{H}_4\text{O}_6$	$\times 5.5$	$\times 6$	$\times 6.5$	$\times 7$	$\times 7.5$	$\times 8$	$\times 8.5$	$\times 9$	$\times 9.5$	$\times 10$
Range in size (μ)	428.4–571.2	285.6–428.4	213.8–428.4	285.6–499.4	—	213.8–356.6	—	213.8–356.6	—	285.6–356.6
Average size (μ)	542.0 ± 28	328.2 ± 4.3	314.1 ± 21	385.0 ± 25.5	—	285.6 ± 18	—	285.6 ± 3	—	314.1 ± 3

Cor. co. (1): -0.3.

Conc. grade	0	1	2	3	4	5	6	7	8	9	10
Conc. of KNO_3	0	$10^{-3} \times 1$	$\times 2$	$\times 3$	$\times 4$	$\times 5$	$\times 6$	$\times 7$	$\times 8$	$\times 9$	$\times 10$
Range in size (μ)	—	—	285.6–856.8	—	571.2–714	357.0–642.2	214.2–499.4	214.2–571.2	357.0–642.2	—	—
Average size (μ)	—	—	456.9 ± 42	—	585.4 ± 22.5	499.4 ± 24	357.0 ± 20	385.0 ± 35	471.0 ± 34	—	—

Cor. co. (2): 0.3.

Conc. grade	11	12	13	14	15	16	17	18	19	20
Conc. of KNO_3	$10^{-2} \times 1.1$	$\times 1.2$	$\times 1.3$	$\times 1.4$	$\times 1.5$	$\times 1.6$	$\times 1.7$	$\times 1.8$	$\times 1.9$	$\times 2$
Range in size (μ)	349.8–524.7	—	408.1–524.7	408.1–524.7	291.5–583	291.5–466.4	291.5–466.4	291.5–583	233.2–583	291.5–524.7
Average size (μ)	542.0 ± 24	—	501.2 ± 19	478.0 ± 15	489.6 ± 36	421.0 ± 29	421.0 ± 26	542 ± 25	408.1 ± 18	419.4 ± 23

Cor. co. (2): -0.8.

As seen in Table 2, the mean obtained in the first experiment was higher than that in the second experiment. A significant increase in size of the perithecium was observed at the three concentration grades 4, 8 and 11.

In the third experiment the residual nitrogen ion in the ammonium minimum medium in culture of either A (+) or a (-) strain was quantitatively measured by the spectrophotometrical method to find out the effect of residual ammonium and nitrate ion in the CFs (Fig. 1).

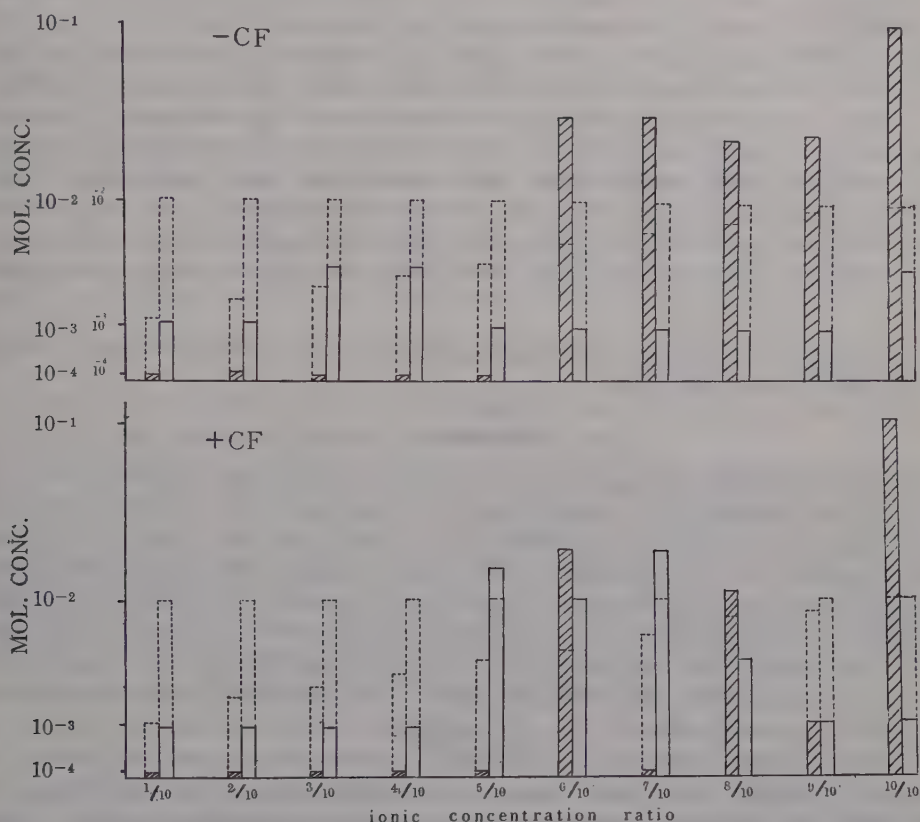





Fig. 1. The residual amount of NH_4^+ and NO_3^- in the CF. The signs,  and , show the residual concentration of NH_4^+ and NO_3^- , and  the initial concentration of both ions.

The residual amount of nitrate in the +CF decreased less than 10 per cent of the initial one at the concentration grades from 1 to 4 and from 8 to 10, while it increased at the grade 7. The residual amount of ammonium decreased at the grades from 1 to 5, 7 and 9, while it increased at the grades 6, 8 and 10. In the -CF the residual amount of nitrate decreased at the all concentration grades from 1 to 10 and that of ammonium increased at the concentration grades from 6 to 10. Both the ions were exhausted when supplied at the lower concentration ratio of these ions. Moreover, it has been reported that the pH change of the culture solution during the growing period of the mating type strain has some influence on an exhaustion of the nutrients⁶). Since it became clear that a specific ratio of ammonium to nitrate in the culture solution is necessary for the perithecial growth as shown in the previous experiment, the pH value of the culture filtrate was measured after 96, 144 and 216 hrs.

The pH value decreased to less than the initial one in 96 hrs. with an increase

Table 3. pH value at various culture durations on the media with the various amount of either ammonium or nitrate. Initial pH; 5.8-6.0.

CF	Conc. grade										
	Culture duration	1	2	3	4	5	6	7	8	9	10
+CF	96 hrs.	6.0	4.2	4.4	4.2	4.2	4.2	3.8	3.8	3.8	4.0
	144	4.9	4.7	4.9	4.9	4.6	4.5	4.7	4.7	4.7	4.5
	216	6.5	6.6	6.2	6.2	3.7	4.7	4.5	4.7	5.5	5.7
-CF	96	6.0	4.2	4.2	4.2	4.2	4.2	3.4	3.4	3.8	4.0
	144	4.7	4.5	4.5	4.3	4.3	4.3	4.5	4.5	4.3	4.3
	216	6.4	6.7	6.8	6.0	6.0	5.9	5.5	5.5	5.5	5.5
±CF	96	4.0	3.6	3.8	3.8	4.0	3.8	3.4	3.4	3.8	4.0
	144	5.1	4.7	4.7	4.7	4.5	4.5	4.7	4.7	4.7	4.7
	216	3.6	3.8	3.7	3.4	3.6	3.4	3.8	4.6	4.6	4.7

of the amount of ammonium in either the +CF or -CF, and then returned gradually to the initial value in 216 hrs.

Discussion

For understanding the significance of both ammonium and nitrate ions for the growth of the perithecia, the present work was designed chiefly to investigate two main points, (1) the pattern of utilization of both NH_4^+ and NO_3^- ions in the media by these strains, and (2) the effect of the culture filtrate obtained from the media containing these ions, on the growth of perithecia. As shown in Table 1-(I), the average diameter of the perithecium in the medium with 10^{-2} M. nitrate ion was larger than in that with 10^{-2} M. ammonium ion. Similarity between the two experimental lots was recognized below the probability 0.05. The ammonium ion as the sole nitrogen source was more effective for the perithecial growth in concentrations below 10^{-2} M. than in those above 10^{-2} M. (Table 2). On the other hand, the nitrate ion was effective evenly over these ranges of concentrations and the its efficacy was almost equivalent to that of ammonium ion at the middle concentration grade. Consequently it may be stated that the ammonium ion may be utilized relatively well for the perithecial growth in the lower concentration range; on the other hand, the nitrate ion may act effectively over a wide range of concentration, but with little effect upon the size of perithecia. The negative correlation coefficient of growth of the perithecium with the amount of ammonium ions was higher than that of nitrate ions. The growth rate of perithecia rose progressively with a decrease in the amount of ammonium ion to zero (Table 1). This fact suggested also that ammonium ions acted in an inhibitory way, while nitrate ions acted productively.

In order to clarify the mechanism of utilization of these ions for the growth of perithecia, the residual amount of these ions and the pH change of the culture media were measured. No correlation was recognized between the perithecial growth revealed at the concentration grades 0.2, 4.5 and 6 in +CF and at the concentration grades 7 and 10 in -CF, and the residual amounts of these ions at these concentration grades. The significance of the ammonium minimum medium for the formation of perithecia lay in the +CF and the -CF, as was shown in a previous paper³). The effect of the ammonium ion on the growth of perithecium seemed to lie in the CFs (Table 1.). The pH value 4.6-4.8 of the medium during the vegetative growth decreased in 96 hrs. and returned to the initial value after 144 hrs. followed by a

slight rise after 216 hrs. In study on the perithecial formation of *Sordaria fimicola*, Lilly and Barnett⁶⁾, have stated that perithecia were formed only after the pH value of the medium had risen above 6 in a medium containing 25g. glucose and casein hydrolysate (equivalent to 20 g. casein), but this pH alone was not sufficient to insure sexual reproduction. Esser⁷⁾ confirmed the increase of pH value of the medium at the time of the spore formation in *Podospora anserina*. Ryan *et al.*⁸⁾ stated that the decrease of the pH value during the growth of mycelia was due to the removal of NH_4^+ , while the pH value rose when only NO_3^- was used for growth. It is reasonable to assume that the decrease of pH value during the early growing stage observed in the author's experiment (Table 3) may depend upon the exhaustion of NH_4^+ ; on the other hand its increase in the later growing stage may due to the utilization of NO_3^- . Therefore, it may be concluded that a small amount of NH_4^+ is used during the early stage of growth, while NO_3^- is used during the later growing stage for the production of some substance which stimulates the perithecial formation.

Summary

The present report deals with the effect of two nitrogen ions, ammonium and nitrate, on the perithecial growth in *Neurospora crassa*.

The growth of perithecia is activated in media with both ammonium tartrate (0.5×10^{-3} M. $\sim 0.5 \times 10^{-2}$ M.) and potassium nitrate (10^{-2} M.) rather than in those with ammonium tartrate (0.5×10^{-2} M.) or potassium nitrate (10^{-3} M. $\sim 10^{-2}$ M.). It is also made evident that the perithecial enlargement should be related to some stimulative substance produced by either A(+) or a(-) strain in the media of the special ionic concentration. Ammonium ions may be utilized in the relatively lower concentration range ($< 10^{-2}$ M.) at the early growing stage, while nitrate ions may act effectively over a wide range of concentration at the later growing stage.

References

- 1) Hirsch, H. M., *Physiologia Plantarum* 7: 72 (1954).
- 2) Ito, T., *Bot. Mag. Tokyo* 69: 369 (1956).
- 3) —, *ibid.* 72: 852 (1959).
- 4) Westergaard, M., and Mitchell, H. K., *Amer. Jour. Bot.* 34: 573 (1947).
- 5) Hawker, E. L., *Camb. monographs in Exp. Biol.* 6 (1957).
- 6) Lilly, V. G., and Barnett, H. L., *Amer. Jour. Bot.* 34: 131 (1947).
- 7) Esser, K., *Compt.-rend. Lab. Carlsberg, Ser. Physiol.*, 26: 7 (1960).
- 8) Ryan, F. G., Beadle, G. W., and Tatum, E. L. *Amer. Jour. Bot.* 30: 784 (1943).

摘 要

伊藤太郎：アカパンカビの子実体形成 IV.

培地中におけるアンモニウムイオンと硝酸イオンの被子器の直径に対する影響

本報は *Neurospora crassa* の被子器生長におよぼす二種の窒素源イオン、アンモニウムならびに硝酸イオンの影響に関する研究報告である。

被子器生長はアンモニウム最小培地（酒石酸アンモニウム 0.5×10^{-3} — 0.5×10^{-2} M. 硝酸加里 10^{-2} M.）で、とくに NH_4^+ と NO_3^- の濃度比が 10:10, 5:10, 3:10 および 0:10 の場合に良好である。この添加窒素源の特定量添加のさいにみられる生長作用は、 NH_4^+ と NO_3^- の特定濃度比を有する合成培地中で A(+) 系および a(-) 系が、それぞれその反対性型系、すなわち、a(-) 系および A(+) 系菌糸に原被子器 protoperithecia 形成を誘起し、さらにその生長を助長する性ホルモン様物質を生成するためであることが明らかとなった。 NH_4^+ は比較的低濃度で成育初期に、 NO_3^- は比較的広い濃度範囲で成育後期に有効に利用されると思われる。（帯広畜産大学酪農学教室）

Physiological and Ecological Analyses of Shade Tolerance of Plants

1. Growth of Green-grams under Varying Light Intensities*

by Nobuo NOMOTO**, Hideo IWAKI*** and Masami MONSI***

Received February 27, 1961

Shade tolerance has long been treated mainly in the field of forestry, where the term *tolerance* itself refers to the ability of plant to survive under deep shade, because of its particular importance for the silviculture purpose, and much practical information has been obtained on the tolerant and intolerant tree species¹). Recently, further ecological or physiological approaches to this subject have been made by a number of workers²). Most of them discussed the shade tolerance on the basis of a comparison among plant species of certain characteristics, the minimal light for survival or the light compensation point of CO₂-gas exchange. It is noticeable that Blackman and Wilson^{3, 4}) and others⁵⁻⁷) have thrown much new light on this problem by analysing the effect of light intensities on plant growth, especially on two attributes, net assimilation rate (NAR) and leaf area ratio (LAR)⁸), and their discussions have been introduced by Kira, Shinozaki and Hozumi into a Japanese text book of ecology⁹) and well appreciated.

The shade tolerance, however, could fundamentally be clarified with analytic-synthetic studies¹⁰⁻¹²) of the growth process or dry matter production of the plant under the given shaded conditions. Monsi¹³), one of the authors, has presented five representative schemata of dry matter reproduction systems of plants and plant communities, and he tried to apply the schemata to an elucidation of difference in the shade tolerance between *Pinus* and *Picea* using old data of Stålfelt¹⁴).

A series of experiments have been designed to clarify the physiological and ecological responses of plants to varying degrees of shade. Special attention has been focused on some factors or characteristics which are principal in dry matter production under shaded conditions. In the present paper, a part of the experimental results obtained in green-grams will be presented and some analyses will be made about the shade tolerance in the light of dry matter production of the plants.

Material and Method

Experiments with the potted young plants of green-gram (*Phaseolus aureus* Roxb = *Ph. viridissimus* Tenore) were performed in the summer of 1955. The experiments were restricted within the early stages of plant growth because of disadvantageous effects of pot culture on root growth and of mutual shading. In each of 180 of earthen pots of ca. 15 cm. in diameter, 30 green-gram seeds selected were sown on August 6, 1955, and the pots were divided into five groups; those of the one group were placed in a full natural daylight and those of the others each under four different intensities of shading. The shade treatment was produced by screening with wooden lattice of

* A part of the expense was supported by a Grant in Aid of Scientific Research of the Ministry of Education.

** Biological Institute, Faculty of Arts and Sciences, Ibaraki University, Mito, Japan.

*** Department of Botany, Faculty of Science, University of Tokyo, Hongo, Tokyo, Japan.

which slits were properly adjusted, so that the relative light intensities under them were respectively to be 75, 50, 30 and 20% of full daylight. Each group of the pots were divided once again into two parts, the one was periodically supplied with a proper amount of Boysen Jensen's culture solution (the N-series) and the other was given the same amount of water as the solution (the O-series). All pots were properly taken care of without suffering from drought. After establishment of seedlings, 15 plants per pot were left to make the sample plant equal in size. Further, on each sampling occasion, 10 plants from two pots (5 plants from a pot on and after Aug. 22) were chosen at random in order to determine the leaves, roots and stems in fresh and dry weight (drying at 85°), and also the leaf area. At that time, subsamples of plants were submitted to the measurements of photosynthetic and respiratory activities.

Results and Discussion

Growth in plant weight: The effects of varying shades on the growth of green-gram plants are set out in Fig. 1a and b, which represent the results for the N- and O-series, respectively.

Green-gram seeds used in this experiment were of 42.7 mg. in mean dry weight, including seed-coat of 4.2 mg. After emergence, the cotyledons reduced their dry weight gradually to 1.8 mg. by August 12 when most of them shed, though they are omitted in Fig. 1.

Depressing effect of shade on plant growth in dry weight was evident from Fig. 1a and b. In the deep shade (20 and 30%), the depression of growth by shading was very marked, while the growth of the lightly shaded plants (75%) followed closely the growth in full light. In the N-series, for example, plant dry weight on September 14

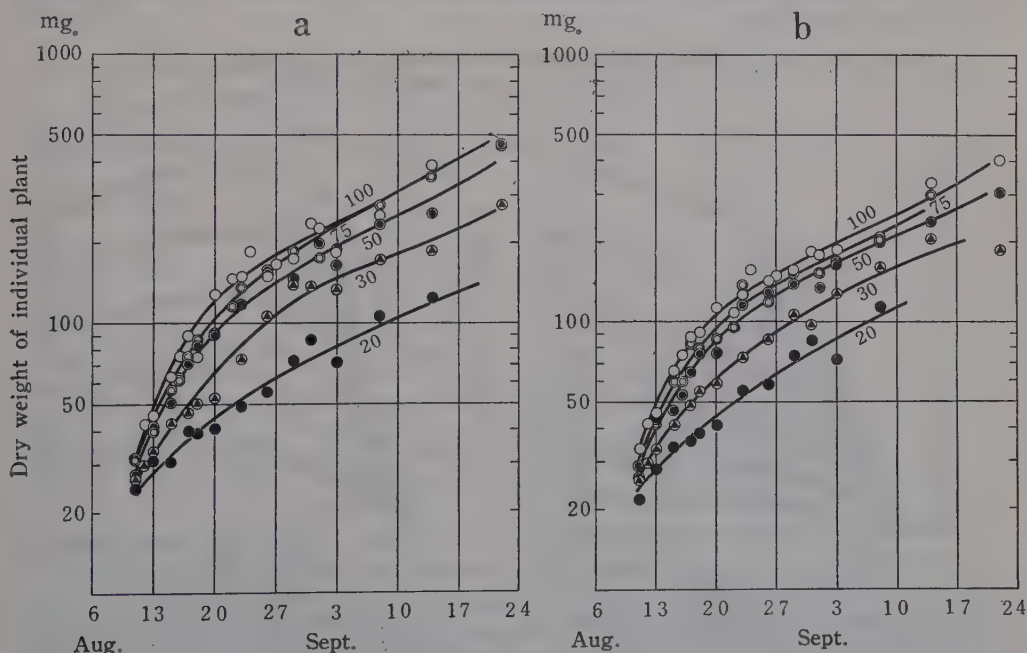


Fig. 1. Growth curves in dry weight of green-gram plant under 100, 75, 50, 30 and 20% daylight. Plants of the N-series (a) were supplied with nutrient solution periodically, while those of the O-series (b) only with water.

(40 days after the sowing) was averaged 384 mg. in full light, 350 mg. in 75% light, 258 mg. in 50% light, 185 mg. in 30% light, and 124 mg. in 20% light, i.e. in relative values 100:93:67:48:32.

It is interesting that significant effect of nutrient supply was observed only in full light and in light shades. In full daylight, growth in dry weight increased by about 10% by the application of nutrient solution. While for the heavily shaded plants (20%), the nutrient supply gave no significant effect on the plant growth, with the result that the growth in plant weight of the O-series showed a similar trend to that of the N-series.

Growth in leaf weight and in leaf area: In the view of the role in the dry matter production, plant organs can be divided into two main components, photosynthetic system (F), and non-photosynthetic system (C)^{13,15}.

In Fig. 2 are presented the growth curves in leaf weight of green-gram plant grown under varying intensities of shading. It appears from this figure that the depression of leaf weight growth by shading was marked particularly in densely shaded plots (30 and 20% light). This trend, which was similar to that of total plant weight growth, was clearly seen both in the N- and O-series, especially in the later stages of the experiments.

Favourable effect by nutrition supply was observed in higher illumination plots. Under 100, 75 and 50% light, leaf weight growth of the N-series, at 6 weeks after the sowing was about 20% higher than that of the O-series but slight difference was seen between the N- and O-series under 30 and 20% daylight.

Since the photosynthetic system (F) generally performs its function as leaf area (\bar{F}) in photosynthesis as well as in acceptance of light, it is quite necessary in analysis of the shade tolerance of plants to consider growth in leaf area with reference to varying light intensities.

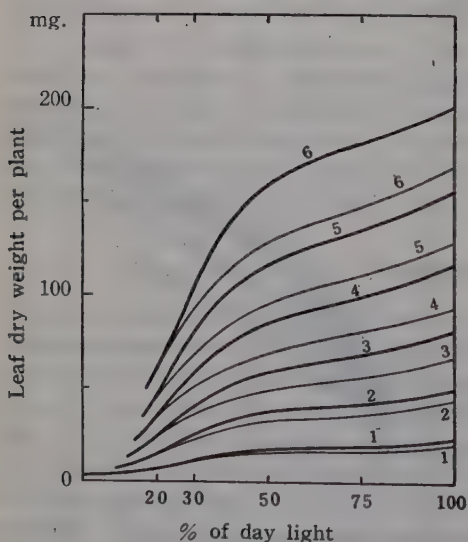


Fig. 2. Mean leaf dry weight of green-gram plant of the N-series (thick line) and O-series (thin line) under varying light intensities. Numerals at the curves indicate number of weeks after the sowing.

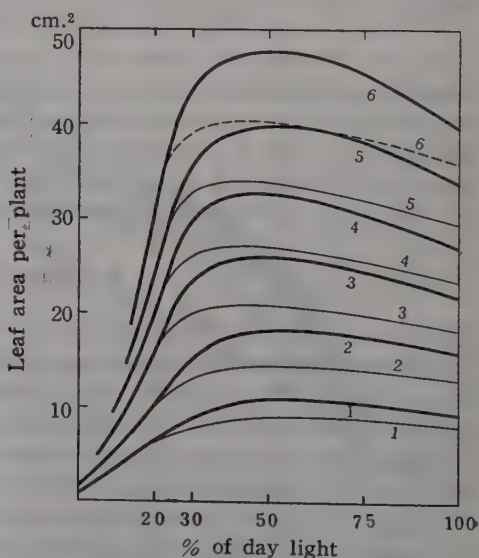


Fig. 3. Mean leaf area of green-gram plant of the N-series (thick line) and O-series (thin line) under varying light intensities.

The effect of shading on the leaf area growth is shown in Fig. 3, where total leaf area of green-gram is plotted against light intensity, at different stages of growth. Under moderate degrees of shading, total leaf area of a plant was larger than in full light, in contrast to the growth in leaf dry weight: optimal leaf area growth was observed, over the whole growing period under 50% light rather than in full light (in the N-series). Heavy shading (30 and 20% light), however, caused remarkable depression of growth in leaf area. This depression is probably due to the deficiency of leaf-forming material by reduced dry matter production under heavy shade. Relations similar to those in the N-series were also found between leaf area and illumination level among the plants of the O-series, though in this series the optimal leaf-area growth was observed under somewhat lower light intensities than those in the N-series.

In full light and light shade the effect of nutrition supply was clearly seen on leaf area growth as well as on leaf weight growth. While, under heavy shade of 20% light, the application of nutrient solution brought about no effect on the leaf growth.

Relative growth rate: Inspection of Fig. 1 indicates that the growth rate of plant weight in early stage (August 13-20) decreased progressively as shading increased. With the advance of the development, however, difference in growth rate caused by varying light intensity became smaller. In later stages, the highest growth rate was observed in light shade (75% light) rather than in full light.

The relative growth rates (RGR, mg./mg./week) of green-gram plants for the period August 13 to September 10 are shown in Table 1. The RGR of green-gram plants in the N-series calculated for the week commencing on August 13 was 0.994, 0.861, 0.784, 0.707 and 0.490 mg./mg./week for 100, 75, 50, 30 and 20% light, respectively. Thus, the RGR under 20% light was only a half of the rate observed under full light.

Table 1. Variation with time of relative growth rate (RGR, mg./mg./w.) of green-gram plants under varying light intensities.

Relative light intensity		Aug. 13-20	Aug. 20-27	Aug. 27-Sep. 3	Sep. 3-10
N-series	100%	0.994	0.397	0.262	0.266
	75	0.861	0.461	0.332	0.266
	50	0.784	0.433	0.317	0.248
	30	0.707	0.508	0.295	0.216
	20	0.490	0.343	0.280	0.238
O-series	100%	0.773	0.368	0.268	0.243
	75	0.759	0.369	0.269	0.256
	50	0.765	0.390	0.262	0.241
	30	0.568	0.405	0.320	0.261
	20	0.452	0.342	0.301	0.269

While the RGR for the period August 27 to September 3 was a little high in light shade (75 and 50% light) and in 30% light compared with that in full light (Fig. 4).

Net assimilation rate and leaf area ratio: As mentioned above, the relative growth rate was expressed by Blackman and Wilson^{3,4}) as the product of net assimilation rate (NAR) and leaf area ratio (LAR).

The NARs (mg./cm²./week) of green-gram plants calculated for a period of 4

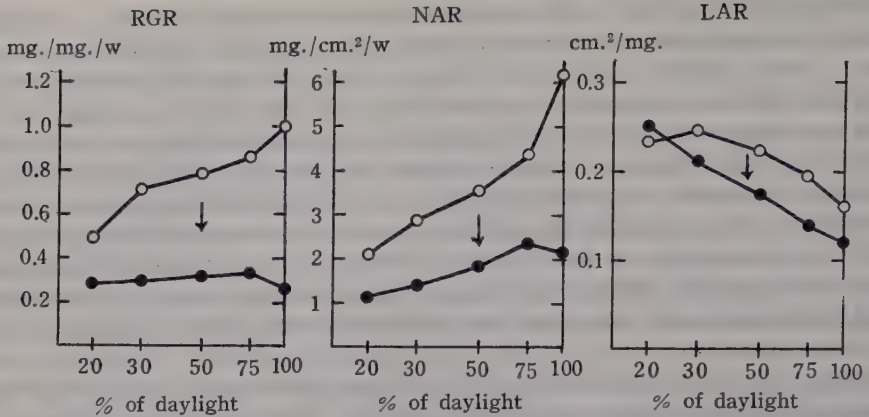


Fig. 4. Relationship of relative growth rate (RGR), net assimilation rate (NAR) and leaf area ratio (LAR) to light intensity. Open circles: in early stage (Aug. 13-20). Closed circles: in late stage (Aug. 27-Sep. 3).

weeks from August 13 to September 10 are shown in Table 2. It is apparent that the NAR generally increased with increasing light intensity and that there was slight difference in NAR between the N- and the O-series. The high NAR must be a positive factor in the dry matter production of the plants that grow under high levels of illumination.

Table 2. Variation with time of net assimilation rate (NAR, mg./cm.²/w.) of green-gram plants under varying light intensities.

Relative light intensity		Aug. 13-20	Aug. 20-27	Aug. 27-Sep. 3	Sep. 3-10
N-series	100%	6.17	3.08	2.17	2.32
	75	4.38	2.93	2.37	2.05
	50	3.52	2.30	1.82	1.52
	30	2.88	2.27	1.39	1.06
	20	2.09	1.41	1.12	0.99
O-series	100%	5.44	2.95	2.20	2.06
	75	4.42	2.50	1.86	1.80
	50	3.99	2.34	1.59	1.48
	30	2.60	1.85	1.49	1.27
	20	2.04	1.41	1.23	1.17

The NAR in the N-series during August 13-20 was 6.17 mg./cm.²/week for full light, and only 2.09 mg./cm.²/week for 20% light. Thus the shading could markedly depress the NAR. The high RGR under full light observed in the early stage was due mainly to the NAR that was high enough to cause rapid growth in plant weight. As the plants grew, however, NAR was reduced progressively in all the plots, and the difference in NAR among the plots differently treated became smaller (Fig. 4). So the extent of the decrease in NAR was naturally large in the high illumination plots.

The leaf area ratio (LAR), the ratio of total leaf area (\bar{F}) to total plant dry weight (\bar{W}), of the green-gram plant is shown in Table 3. The LAR values tended

Table 3. Variation with time of leaf area ratio (LAR, cm.²/mg.) of green-gram plants under varying light intensities.

Relative light intensity		Aug. 13-20	Aug. 20-27	Aug. 27-Sep. 3	Sep. 3-10
N-series	100%	0.161	0.129	0.121	0.114
	75	0.196	0.157	0.140	0.129
	50	0.223	0.189	0.174	0.163
	30	0.246	0.224	0.212	0.203
	20	0.233	0.243	0.251	0.240
O-series	100%	0.142	0.124	0.122	0.118
	75	0.172	0.148	0.145	0.142
	50	0.192	0.167	0.164	0.163
	30	0.219	0.219	0.215	0.206
	20	0.221	0.243	0.245	0.230

to be small with increasing light intensity. For the period after August 20, under full light the LARs were only about a half of the values in deep shade of 20% light. This factor will bring about a positive effect on the dry matter production of the plants growing under shade. The same trend was observed in the O-series as well as in the N-series.

As plants grew, a slight decline in the LAR was seen in full light, but the extent of the decline was markedly small compared with the case of the NAR. As the results, the relation between the LAR and the light intensities could be held almost constant over all of the experimental periods.

As stated above, the relation between the RGR and shading can be analysed by the relation of light to the NAR and to the LAR. In the early stage (August 13-20), the relation of the NAR to light was more important for the RGR. The NAR was very high in full light compared with that under heavy shade, with the result that the RGR was the highest for 100% light and the lowest for 20% light.

In later stages, however, the difference in the NAR among the plots with different shading treatments tended to be small and the relation between the LAR and the light intensity became important for the RGR. Thus, the highest RGR value was observed in light shade (75 and 50% light) rather than in full daylight.

The NAR is a complicated factor, as shown by Iwaki¹⁶), which can be further analysed into the components, such as light intensity received by plant, photosynthetic ability of each leaf, respiration intensity of each organ, and C/F ratio, etc. Effects of shading on the photosynthetic ability and the respiration intensity or on the C/F ratio of the growing plant will be discussed in the succeeding paper.

The ratio of leaf area to leaf weight: It is evident from Fig. 4 and Table 3 that the LAR became higher as shading increased. But this indicates by no means a fact that the proportion of leaf dry weight to total plant dry weight was large under shade compared with that under full light.

As the LAR (\bar{F}/W , cm.²/mg.) can be expressed by the product of the leaf weight/total plant weight ratio (F/W , mg./mg.) and the leaf area/leaf weight ratio (\bar{F}/F , cm.²/mg.), it is necessary to know the responses of the two ratios to varying levels of shading.

Table 4 shows the leaf weight/total plant weight ratio (F/W). It is apparent that

Table 4. Effect of light intensity on the leaf weight/total plant weight (F/W, mg./mg.) ratio of green-gram plants.

Date	Series	Relative light intensity				
		100%	75%	50%	30%	20%
Aug. 17	N	0.472	0.413	0.388	0.368	0.359
	O	0.483	0.413	0.441	0.386	0.357
Aug. 26	N	0.470	0.425	0.422	0.382	0.364
	O	0.445	0.413	0.407	0.351	0.369
Sep. 8	N	0.513	0.439	0.472	0.439	0.399
	O	0.455	0.505	0.441	0.430	0.388

Table 5. Effect of light intensity on the leaf area/leaf dry weight (\bar{F}/F , cm.²/mg.) ratio of green-gram plants.

Date	Series	Relative light intensity				
		100%	75%	50%	30%	20%
Aug. 17	N	0.307	0.459	0.500	0.667	0.695
	O	0.264	0.354	0.418	0.550	0.582
Sep. 8	N	0.242	0.286	0.365	0.452	0.578
	O	0.240	0.324	0.340	0.465	0.633

the proportion of leaf weight was reduced by shading, though the difference in the F/W ratio was rather small, thus, in other words, the proportion of non-photosynthetic organs (C) to the total plant weight increased with shading. For example, on August 17, the F/W ratio of the N-series was 0.472 in full light and 0.359 under 20% light.

A comparison of the F/W ratio between the N- and the O-series shows that the effect of nutrient supply was not significant at each of the five illumination levels and at different stages of the experiment (August 17, 26 and September 8).

A marked effect of shading was also observed on the leaf area/leaf weight ratio

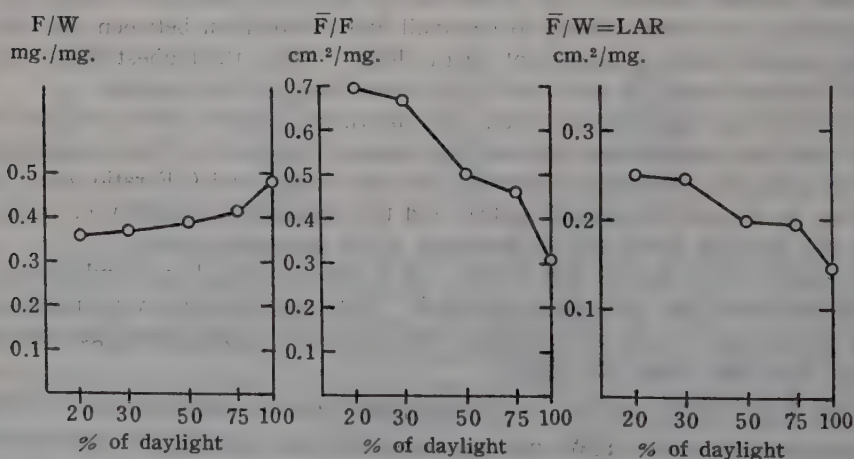


Fig. 5. Relationship of leaf weight/total plant weight (F/W) ratio, leaf area/leaf weight (\bar{F}/F) ratio and leaf area ratio ($\bar{F}/W=LAR$) to light intensity. All the values were calculated from the data measured on August 17.

(\bar{F}/F), which is the reciprocal of the leaf dry matter index presented by Totsuka and Monsi¹⁶). The \bar{F}/F ratio of green-gram plants decreased with increasing illumination: the values of the N-series on August 17 being 0.307, 0.459, 0.500, 0.667 and 0.695 cm.²/mg. for 100, 75, 50, 30 and 20% light, respectively. Thus, the expansion of leaf area under deep shade (20% light) was, at the same expense of dry matter, more than twice as large as that under full light.

Furthermore, the \bar{F}/F ratio was not affected by nutrition level, as shown in Table 5, where the ratio of the N-series to compared with that of the O-series at different levels of shading.

Although the F/W ratio tended to be large with increasing illumination, the extent of the variation by shading was rather small for green-gram plants, while, the \bar{F}/F ratio, in contrast to the F/W ratio, increased markedly by shading. The positive effect of shading on the \bar{F}/F ratio was much greater than the depressing effect on the F/W ratio, with the result that the LAR, which is expressed by the product of the F/W ratio and the \bar{F}/F ratio, became larger as shading increased (Fig. 5). This may bring about an advantageous effect on the dry matter production under shade. Thus, the high value of the \bar{F}/F ratio is one of the important factors for plants to survive or to make positive dry matter production under deep shade. The ability of plants to make the ratio of \bar{F}/F high under shade plays no doubt a positive role in the shade tolerance.

Summary

In order to analyse the shade tolerance of plants on the basis of dry matter production, the growth of green-gram plants was examined under varying light intensity (full daylight, 75, 50, 30 and 20% light) in the early stages of growth, and their responses to shading were determined.

1. The growth of total plant weight was depressed very markedly by heavy shading (30 and 20%), while the growth in 75% light followed closely that in full light.

2. The leaf growth decreased in dry weight with increasing shading, but it showed in area an optimum in 50% light rather than in full light. Heavy shading caused remarkable depression of leaf growth not only in weight but also in area.

3. The relative growth rate (RGR) increased with increasing light intensity in the early stage of the experiment (August 14-20), while in the late stage (August 27-September 3) the RGR was a little higher in 75, 50 and 30% light than in full light.

4. The net assimilation rate (NAR) generally decreased with increasing degree of shading. As the plants grew, the NAR was reduced progressively in all the plots with diminished plot differences.

5. The leaf area ratio (LAR) tended to be small with increasing light intensity. The ratio of leaf weight to total plant weight (F/W) was decreased a little by shading, while the ratio of leaf area to leaf weight (\bar{F}/F) increased markedly, especially in the deep shade. Thus the LAR, which was expressed by the product of F/W and \bar{F}/F , became larger as shading increased.

6. Positive effect of nutrient supply was observed, only in full light and in light shade, on the growth in plant weight, leaf weight and leaf area. On the F/W and \bar{F}/F ratio, however, there was no significant effect of nutrient supply.

References

- 1) Harada, Y., Rep. Hokkaido For. Exp. Stn., Imp. For. Bu. No. 1: 1(1942) (Jap.). 2) Baker, F. S., Principles of silviculture, New York, 60 (1950). 3) Blackman, G. E., and Wilson, G. L., Ann. Bot., N. S. 15: 63 (1951). 4) —, and —, ibid. 15: 373 (1951). 5) —, Black, J. N., and Kemp, A. W., ibid. 19: 527 (1955). 6) —, and Black, J. N., ibid. 23: 51 (1959). 7) Maggs, D. H., ibid. 24: 434 (1960). 8) Watson, D. J., Advances in Agronomy 4: 101 (1952). 9) Kira, T., Plant Ecology No. 2, 244 (1960) (Jap.). 10) Boysen Jensen, P., Bot. Tidskr. 36: 219 (1919). 11) —, Die Stoffproduktion der Pflanzen, Jena (1932). 12) Monsi, M., and Saeki, T., Jap. J. Bot. 14: 22 (1953). 13) —, Bot. Mag. Tokyo 73: 81 (1960). 14) Stålfelt, M. G., Medellandeh från Statens Skögsforsöksanstalt 18: 221 (1921). 15) Iwaki, H., Jap. J. Bot. 16: 210 (1958). 16) Totsuka, T., and Monsi, M., Bot. Mag. Tokyo 73: 14 (1960).

摘 要

野本宣夫*・岩城英夫**・門司正三**：耐陰性の生理学・生態学的解析

1. 種々の強さの光のもとでのヤエナリの生長

植物の耐陰性を物質生産の面から解析するため、ポット植えのヤエナリを、それぞれ 100, 75, 50, 30, 20% の自然光下におき、その生長におよぼす光の強さの影響をしらべた。

1. 光不足による個体重生長の低下は、20%・30% 光下でいちじるしかった。75% 光下のヤエナリの生長は、全光下とほとんど差がなかった。

2. 葉重生長は、個体重と同様に、光の低下とともに減少したが、葉面積生長の最大は、全光下よりもむしろ 50% 光のもとでみられた。30%・20% 光のもとでは、葉重・葉面積の生長低下がいちじるしかった。

3. 相対生長率 (RGR) は、実験初期 (8 月 13 日～20 日) には、光の減少に伴なって低下したが、実験後期 (8 月 27 日～9 月 3 日) には、全光下よりも、75, 50, 30% 光下のほうが高かった。

4. 純同化率 (NAR) は光が弱いほど小さくなる傾向を示した (実験初期には全光下で $6.17 \text{ mg./cm.}^2/\text{week}$, 20% 光下で $2.09 \text{ mg./cm.}^2/\text{week}$)。生育が進むにつれて純同化率は全般に低下し、実験区間の差も小さくなった。

5. 葉面積比 (LAR) は、光が弱いほど大きくなる傾向がいちじるしかった。葉重/全重量の比は暗い条件下ではやや小さいが、葉面積/葉重の比は、逆に、いちじるしく大きくなる。このため、両比の積である葉面積比は暗い条件下で大きくなることが明らかにされた。

6. ヤエナリの個体重、葉重、葉面積生長に対する栄養塩類供給の促進的な影響は、明るい条件下では若干みられたが、20% 光下ではほとんどみられなかった。葉重/全重量の比、葉面積/葉重の比に対する栄養塩類供給の影響は認められなかった。(* 茨城大学文理学部生物学教室, ** 東京大学理学部植物学教室)

Chromosome Numbers of Some Japanese Ferns (II)

by Siro KURITA*

Received May 26, 1961

Since I. Manton¹⁾ published a monumental work "Problems of cytology and evolution in the Pteridophyta" in 1950, studies in this field have made a rapid progress. In Japanese ferns, however, the chromosome numbers have been determined only in a few species. After the author's previous reporting in 1960²⁾, he has studied the chromosome numbers in ten more species of Japanese ferns. The results are reported in this paper.

Materials and Methods

Very young fertile fronds were collected in the field or in the garden, fixed in a mixture of three parts of absolute alcohol to one part of glacial acetic acid for about 24 hours, and squashed in the aceto-carmin after Manton¹⁾. All text figures in this paper are inked and bleached photographs. The localities where the plants have been collected are mentioned under each species named.

Results and Discussion

1. *Ctenitis maximowicziana* (Miq.) Ching: Plants from Mt. Ogasa (Shizuoka Pref.) and from Mt. Amagi (Fuji-Hakone-Izu National Park, Shizuoka Pref.) were examined. Forty-one bivalents were observed in meiosis of a spore mother cell (Figs. 1 and a). As the figures show, the size of a bivalent of this species is comparatively small. In a young sporangium there are 16 spore mother cells which give rise to 64 mature spores. Therefore this species is a normally reproductive diploid. Previous studies on the genus *Ctenitis* were made by Manton²⁾ and by Loyal³⁾. They also found the gametic chromosome number of 41 and the former author concluded that the number 41 is the basic chromosome number of the genus *Ctenitis*.

2. *Dryopteris tokyoensis* (Makino) C. Chr.: Plants used were obtained from Mt. Bandai (Asahi-Bandai National Park, Fukushima Pref.) in November, 1958 and planted in the garden. Cytological studies were made in the following summer. Forty-one bivalents were observed in meiosis of a spore mother cell as shown in Fig. 2 and d. In the summer of 1960, fixed fertile fronds of this species from Gotemba (Shizuoka Pref.) were given by Assist. Prof. Y. Shimura of Shizuoka University. These also were found to have 41 bivalents. In both specimens, there were 16 spore mother cells in a sporangium and each of the sporangia put out 64 mature spores. The gametic chromosome number of 41 is well known as the basic chromosome number of the genus *Dryopteris*^{1,2,4,5,6,9)}, so this species is a normally reproductive diploid.

3. *Dryopteris polylepis* (Fr. et Sav.) C. Chr.: Plants were collected at Mt. Asahi (Shizuoka Pref.) and at Mt. Buko (Saitama Pref.). Forty-one bivalents were observed clearly in a spore mother cell as shown in Figs. 3 and e. The number of spore mother cells was found to be 16 and that of mature spores 64. So this species is also a

* Ikeshinden High School, Hamaoka, Shizuoka Prefecture, Japan.

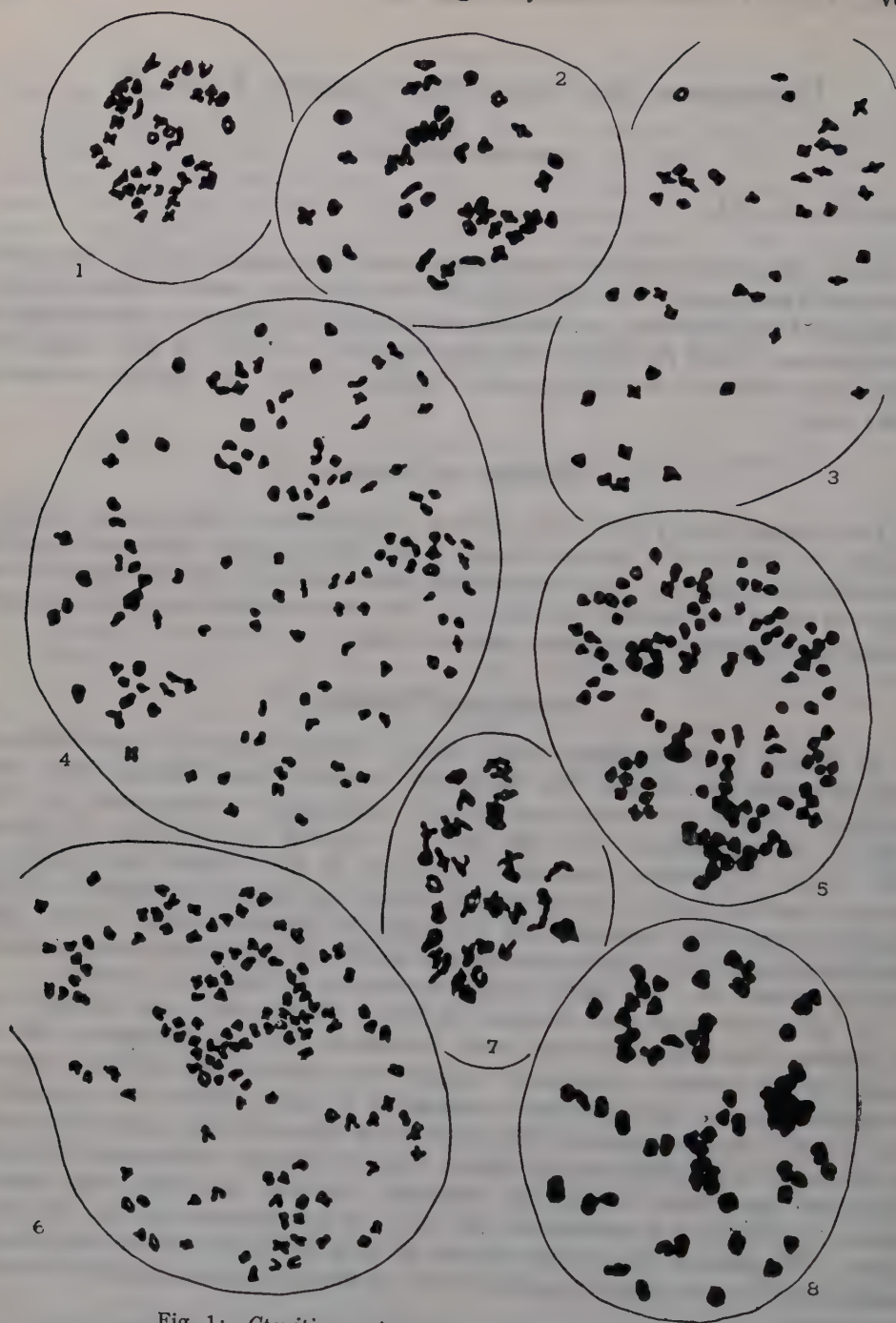
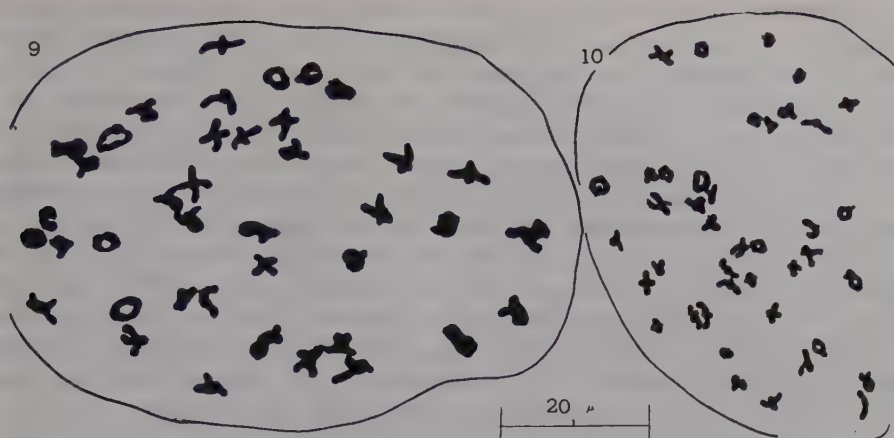


Fig. 1: *Ctenitis maximowicziana*, $n=41$.
 Fig. 2: *Dryopteris tokyoensis*, $n=41$.
 Fig. 3: *D. polylepis*, $n=41$.
 Fig. 4: *D. chinensis*, ' n '=123.
 Fig. 5: *D. erythrosora*, ' n '=123.
 Fig. 6: *D. fuscipes*, ' n '=123.
 Fig. 7: *Lastrea subochthodes*, $n=36$.
 Fig. 8: *L. oligophlebia* var. *subtripinnata*, $n=6-261$.

Fig. 9: *Matteuccia orientalis*, $n=40$.Fig. 10: *Woodsia polystichoides*, $n=41$.

normal diploid.

4. *Dryopteris erythrosora* (Eaton) O. Kuntze: *D. erythrosora* is one of the most common and a very plastic species. Plants from Kikugawa and Hamaoka (Shizuoka Pref.) were studied. The meiotic metaphase of a spore mother cell is shown in Figs. 5 and g. These show 123 bivalents. The number of spore mother cells and of mature spores was counted and it became clear that this species has 8 spore mother cells which give rise to 32 spores. These results suggest that the reproduction of this species is apogamous¹). The sporophytic chromosome number of this species was also counted to be approximately 123 in the root-tip cells. Therefore, so far as the materials examined at the present investigation are concerned, it is sure that this species is an apogamous one.

5. *Dryopteris fuscipes* C. Chr.: *D. fuscipes* is very closely related to *D. erythrosora* but it is distinguished from *D. erythrosora* by its rotundate pinnae. Plants from Hamaoka and Mt. Ogasa (Shizuoka Pref.) were studied. Several spore mother cells showing meiotic metaphase were observed and 123 bivalents were clearly counted as shown in Figs. 6 and i. There are 8 spore mother cells which give rise to 32 mature spores in a sporangium. Therefore the reproduction of this species may be apogamous.

6. *Dryopteris chinensis* (Back.) Koidz.: Plants used were collected at Kikugawa and Hamaoka (Shizuoka Pref.). In spore mother cells, 123 bivalents were observed at meiotic metaphase and at diakinesis. One of such spore mother cells is shown in Figs. 4 and h. Eight spore mother cells and 32 mature spores were observed, too. So this species may be apogamous.

7. *Lastrea subochthodes* (Ching) Tag.: Plants from Fall Shiraito (Shizuoka Pref.) were examined. As shown in Figs. 7 and c, thirty-six bivalents were observed in a spore mother cell. This species yields 16 spore mother cells which give rise to 64 mature spores, so the reproduction of this species may be normal.

8. *Lastrea oligophlebia* (Baker) Copel. var. *subtripinnata* (Tag.) Ohwi: Plants used were collected at Kikugawa (Shizuoka Pref.). A probable 62 bivalents were observed as shown in Figs. 8 and j. All spore mother cells which were studied showed a single large nucleolus and some bivalents (3-5) adhering to the nucleolus. The reproduction of this species may also be normal.

9. *Matteuccia orientalis* (Hook.) Trev.: Plants from Kikugawa (Shizuoka Pref.) were studied. A large number of preparations at meiosis were obtained. In all the preparations, 40 bivalents were apparent as shown in Figs. 9 and f. The same gametic chromosome number was reported by Okuno⁷⁾. The reproduction of this species may be normal, because 16 spore mother cells give rise to 64 mature spores.

10. *Woodsia polystichoides* Eaton: Manton¹⁾ and Britton⁴⁾ examined *W. ilvensis* (L.) R. Br. and they observed approximately 41 chromosomes at meiotic metaphase. Moreover, Manton¹⁾ reported that *W. alpina* has the gametic chromosome number of ca. 82. While, the author investigated *W. polystichoides* which was collected at Ohma (Shizuoka Pref.) and at the shores of Lake Kawaguchi (Yamanashi Pref.) and he found that this species has the gametic chromosome number of 41 as shown in Figs. 10 and b. Hence, in the author's opinion, the number 41 may be the basic chromosome number of the genus *Woodsia*. The reproduction of *W. polystichoides* may also be normal, because 16 spore mother cells give rise to 64 mature spores.

Summary

Ten species of the Japanese ferns were examined to determine the chromosome number. The results are summarized in Table I.

Table I. Chromosome numbers of ten species of the Japanese ferns.

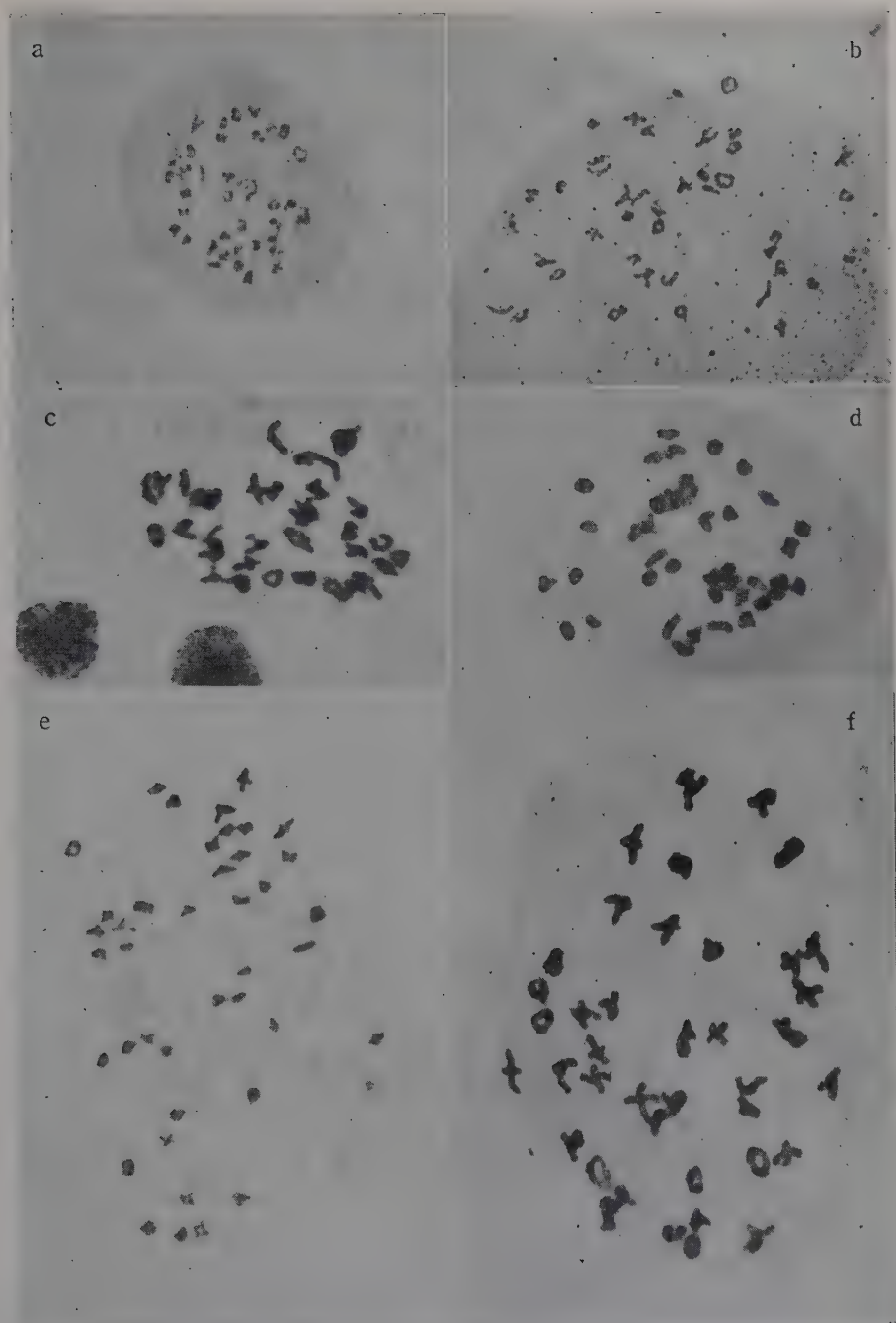
Name of species*	Gametic chromosome number	Number of spores
<i>Otenitis maximowicziana</i> (Miq.) Ching	41	64
<i>Dryopteris tokyoensis</i> (Makino) C. Chr.	41	64
<i>D. polylepis</i> (Fr. et Sav.) C. Chr.	41	64
<i>D. erythrosora</i> (Eaton) O. Kuntze	123	32
<i>D. fuscipes</i> C. Chr.	123	32
<i>D. chinensis</i> (Baker) Koidz.	123	32
<i>Lastrea subochthodes</i> (Ching) Tagawa	36	64
<i>L. oligophlebia</i> (Baker) Copel. var. <i>subtripinnata</i> (Tag.) Ohwi	61-62	64
<i>Matteuccia orientalis</i> (Hook.) Trev.	40	64
<i>Woodsia polystichoides</i> Eaton	41	64

* After the taxonomic nomenclature of Ohwi⁸⁾.

The author wishes to express his deep gratitude to Prof. H. Ito of Tokyo University of Education for identification of the materials and to Assist. Prof. R. Ueda for his helpful guidance and suggestions. He is also grateful to Assist. Prof. Y. Shimura of Shizuoka University for providing the materials for the present study.

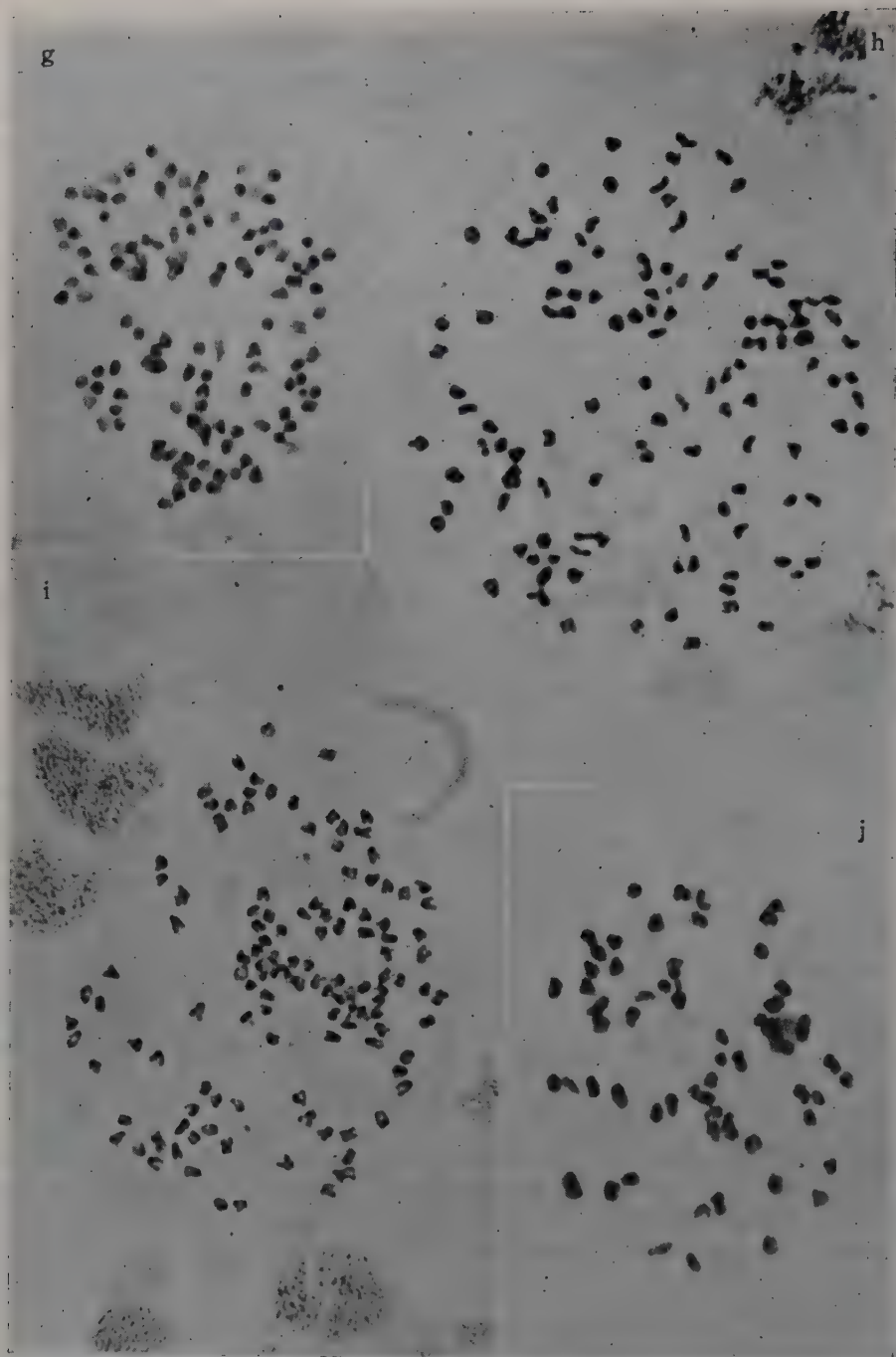
References

- 1) Manton, I., Problems of cytology and evolution in the Pteridophyta, Cambridge University Press (1950).
- 2) —, S.E.B. Symposia **7**: 174 (1953).
- 3) Mehra, P.N., and Verma, S.C., Ann. Bot. N.S. **21**: 454 (1957).
- 4) Britton, D.M., Amer. Jour. Bot. **40**: 575 (1953).
- 5) Manton, I., and Walker, S., Nature **171**: 1116 (1953).
- 6) Wagner, W.H. Jr., Rhodora, **57**: 219 (1955).
- 7) Okuno, S., Bot. Mag. Tokyo **50**: 332 (1936).
- 8) Ohwi, J., Flora of Japan—Pteridophyta Shibundo, Jap. (1957).
- 9) Kurita, S., Jour. Jap. Bot. **35**: 269 (1960).



a: *Ctenitis maximowicziana*, $n=41$.
 c: *Lastrea subochthodes*, $n=36$.
 e: *Dryopteris polylepis*, $n=41$.

b: *Woodsia polystichoides*, $n=41$.
 d: *Dryopteris tokyoensis*, $n=41$.
 f: *Matteuccia orientalis*, $n=40$.



g: *Dryopteris erythrosora*, 'n'=123.

i: *D. fuscipes*, 'n'=123.

h: *D. chinensis*, 'n'=123.

j: *Lastrea oligophlebia* var.
subtripinnata, n=62-61.

摘 要

栗田子郎： シダ類数種の染色体数 (II)

日本産シダ類 10 種の染色体数を報告する。観察の結果、イブキシダは $n=36$ 、イヌガンソクは $n=40$ 、キョスミヒメワラビ・イワデンダ・ミヤマクマワラビ・タニヘゴの 4 種は $n=41$ 、ミドリヒメワラビは $n=61-62$ 、ベニシダ・マルバベニシダ・ミサキカグマの 3 種は $n=123$ であることが判明した。また、孢子母細胞および孢子の数の算定より、ベニシダ・マルバベニシダ・ミサキカグマの 3 種はアボガミーをおこなうものと推測された。とくに、筆者の観察したベニシダでは根端細胞においても約 123 の染色体がみられたので、この種がアボガマス (apogamous) なものであることはまちがいないと考える。

本論文中の学名は大井著、日本植物誌・シダ編のものである。(静岡県立池新田高校生物教室)

Further Studies on the Double-leaf Formation in *Sesamum indicum* L.

by Jun HANAWA*

Received June 9, 1961

It is known that double-leaves sometimes appear in some plants when the phyllotaxis changes from one system to another, for example, from the whorled system to the alternate one or from the decussate system to the alternate one (Fujita, 1949¹). Double-leaves were also induced experimentally in the course of study on phyllotaxis by Snow and Snow²⁻⁵). The present author obtained some double-leaves in *Sesamum indicum* L. by splitting the shoot apex of the dormant embryo⁶). Causes and processes of the double-leaf formation may not be always the same in those different cases. But it may be said that the double-leaf formation takes place commonly as a result of certain disturbances in morphogenetic functions of the shoot apex. In the present paper an analysis of the double-leaf formation in the incised apex will be made from such a viewpoint.

It was previously found in *Sesamum indicum*⁷) that few double-leaves were formed when the splitting of the shoot apex was made 24 hours after sowing, while they were often formed by the operation on the dormant shoot apex. In the former case, normal opposite leaves were formed instead of double-leaves. Therefore the double-leaf formation in this plant seems to be a characteristic morphogenesis of the shoot apex of a dormant embryo, resulting from its certain characteristic response to the operation. It seems therefore that the response of the 24-hour shoot apex to the operation may be different from that of the dormant apex, and the operation is no longer effective for the double-leaf formation on the 24-hour apex. This should be due to the difference of the stages of growth of the shoot apex. It is necessary to determine, first, at what stage of growth of the shoot apex within 24 hours after sowing the responsibility to the operation is lost, and secondly, how the response of the dormant shoot apex to the operation differs from that of the 24-hour shoot apex. It is appropriate in practice to put the latter question in another way, as follows: what difference can be seen between the regeneration processes of those shoots?

The present study deals with the two questions mentioned above. Informations about those points may make it possible to present further explanation than that given in a previous paper⁸) on the cause and process of the double-leaf formation.

Material and Methods

The shoot apex of the embryo of *Sesamum indicum* L. was split longitudinally into halves, with a microscalpel made of a piece of thin razor blade, in a plane running through the two opposite first leaf primordia. The operation was made at intervals of 3 hours during 24 hours after the seeds were soaked in water. Embryos and seedlings were allowed to grow at 28-30° under uninterrupted illumination at

* Department of Biology, Faculty of Science, Tokyo Metropolitan University, Setagaya, Tokyo, Japan.

6,000 lux with fluorescent lamps. The observation was done about the external morphology of the first leaves 10 to 11 days after the operation, and their various forms were recorded. On the other hand, histological preparations of the shoot apex were made at various stages during 24 hours after sowing in order to check developmental changes in it. And one more set of histological preparations was made with the regenerating apices after the operation. Materials were fixed with formalin-acetic acid-alcohol, sectioned at 8 microns in thickness, and stained with Delafield's hematoxylin.

Results

1. *Relationship between the operation stages and the rate of double-leaf formation.*

After the operation, the halved apical meristems regenerated new shoots, and twin-seedlings were formed. The first leaf developing on each shoot took various forms, as shown in Fig. 1. The occurrence rate of each of the forms differed with operation stages. Although there were, as will be described later, found all the intermediate forms, they were classified into three types for convenience of recording. Those are the opposite-leaf, double-leaf and single-leaf types. The opposite-leaf type includes not only normal opposite leaves, but also leaves that approached to each other but remained separate (Figs. 1A and B). The double-leaf type includes the leaves in which two leaves are fused to various extent but two laminae are discernible (Figs. 1C-F). The single-leaf type includes the leaves in which the lamina looks quite single (Fig. 1G). These types are symbolized as Op-, D- and S-type, respectively.

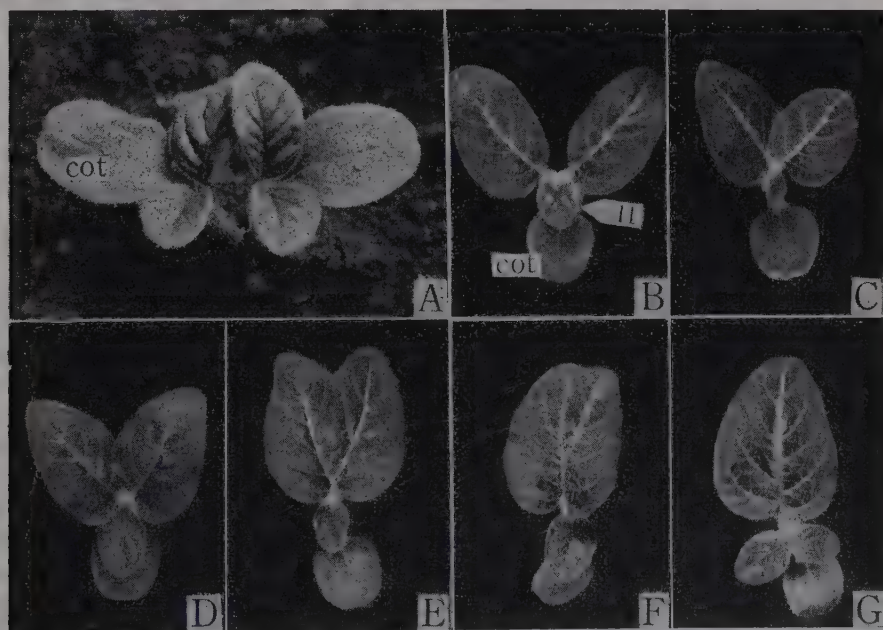


Fig. 1. Various forms of the first leaves that developed after the operation of the shoot apex of embryo. A shows a twin-plant, and B-G one of two shoots of a twin-plant. Cot: cotyledon. II: the second leaf. A, ca. $\times 3$. B-G, ca. $\times 1.5$.

The three leaf types occurred in various combinations on two shoots of the twin-seedling. Kinds of the combination are shown in the left column of Table 1. The combination D-D signifies that two shoots of the twin-plant both bear a double-leaf; D-S means that one shoot of the twins bears a double-leaf and the other a single-leaf, etc. The symbol Nu in the table stands for null and signifies that no leaf was formed, that is, shoot regeneration did not occur. In this way, the occurrence numbers of type combinations at each operation stage are given in Table 1.

Table 1. Occurrence number of type combinations on twin-plant in relation to the operation stages. D: double-leaf type. S: single-leaf type.

Op: opposite-leaf type. Nu: no leaf.

Combinations of leaf types on twin-seedlings	Operation stages							
	0h.	3h.	6h.	9h.	12h.	15h.	18h.	24h.
D—D	1	2	8	4	7	1	0	0
D—S	13	6	9	5	8	1	0	0
D—Nu	10	2	15	4	0	0	0	0
S—S	2	3	7	0	0	0	0	1
S—Nu	2	2	7	0	0	2	1	0
Nu—Nu	3	0	6	2	0	1	1	0
S—Op	3	1	2	13	8	6	10	6
D—Op	0	2	2	12	37	4	12	7
Nu—Op	6	0	11	6	7	5	16	8
Op—Op	0	0	1	4	21	10	63	31
Total number	40	18	68	50	88	30	103	53

It seems necessary, next, to simplify the kinds of leaf type combinations in order to clarify the relationship between the operation stage and the effect of the operation.

Comparing various forms of the first leaves, it was found that they could be put in order according to the degree of fusion. As shown in Fig. 1, there are intermediate forms between the opposite leaf and the double-leaf, and the degree of fusion of the double-leaf gradually increases until a single-leaf is formed. Therefore, the single-leaf can be regarded as an extreme form of the double-leaf. That the single-leaf is not mere one of two leaves of the first pair is decided by the following facts. First, its insertion position is opposite to the cotyledon, not in the intercotyledonary plane as is the case with the normal first leaves. Secondly, its shape is wider than a usual leaf of the first pair. Moreover, the decision is supported by comparing the vascular system of the single-leaf with that of the double-leaf. A and B in Fig. 2 are the cross sections of the petiole and the insertion region of a double-leaf, respectively. In this petiole, two sets of normal vascular system are included, and in the insertion region

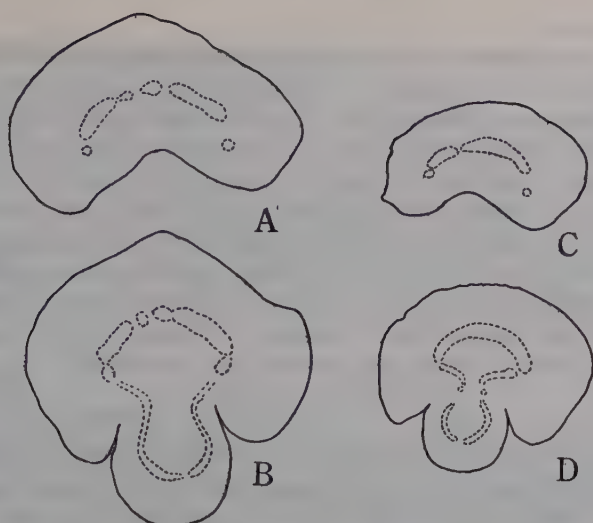


Fig. 2. A and B, transverse sections of the petiole and the insertion region, respectively, of a double-leaf. C and D, transverse sections of the petiole and the insertion region, respectively, of a single-leaf. $\times 33$.

there is a large leaf gap formed by fusion of two gaps. C and D in Fig. 2 are the sections of the petiole and the insertion region of a single-leaf, respectively. This petiole is wider than that of a normal leaf, and contains a wide vascular bundle that appears to be formed by union of two. The leaf gap is also wider than normal.

For the foregoing reason, the D-type and the S-type leaves can be regarded as those of the same category. Accordingly, the combinations D—D, D—S, S—S, D—Nu and S—Nu can be classified as one and the same category, D-group. On the other hand, Op—Op and Op—Nu are included in the Op-group. The latter group is the opposite category to the D-group, if the effect of the operation is judged only from the viewpoint of formation or non-formation of the double-leaf. Then, Op—D and Op—S form

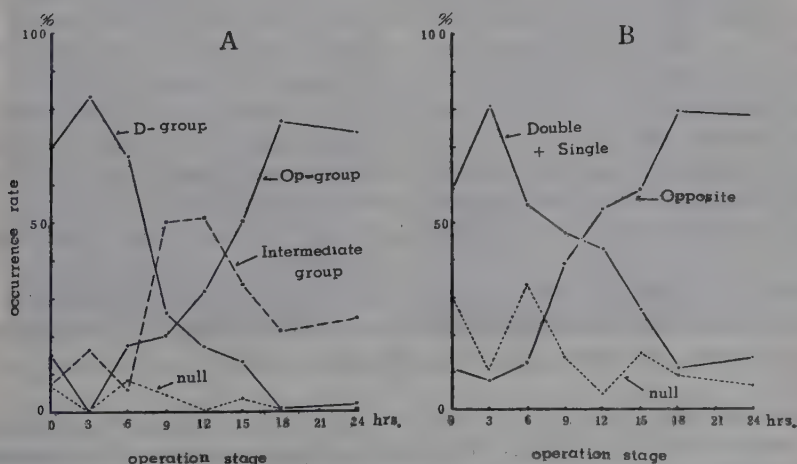


Fig. 3. A, occurrence rate of type combinations on a twin seedling. B, occurrence rate of leaf types.

an intermediate group.

In this way, it is possible to give simple figures indicating a relationship between the operation stage and the effect of the operation. In Fig. 3A, the occurrence rates of above-stated three groups of leaf types, translated from Table 1, are plotted. In Fig. 3B, on the contrary, are plotted the occurrence rates of the leaf types on separate shoots apart from the combination on a twin-seedling.

From Fig. 3, it is evident that the leaves of D-group are formed mainly in 6 hours after sowing, but thereafter decrease rapidly in their formation rate, and are scarcely formed later than 15 hours after sowing. On the contrary, it is clearly shown that the opposite leaves are very few till 6 hours, then increase rapidly, and later than 15 hours they are formed most frequently. Therefore it is concluded that the operation between 6 and 15 hours after sowing had no effect to induce the double-leaf formation.

2. *Cytohistological changes in the shoot apex during 24 hours after sowing.*

Cytohistological changes in the shoot apex during 24 hours after sowing were studied in order to find some relations between the developmental changes in the shoot apex during the experimentation period and the changes of the operation effect just described above.

Dormant shoot apex (Fig. 4A): The shoot apex of a mature dormant embryo is only a small meristematic region lying between the cotyledons. Two primordia of the first foliage leaves are situated oppositely on both shoulders of the shoot apex, and the narrow, rather concave shoot apex proper lies between them. Histological construction of the shoot apex is not fully developed yet. Its complete zonation and stratification are established several days after germination.

In the cells of the leaf primordia as well as of the shoot apex proper, cytoplasm stains intensely, but reserve materials (aleurone grains) are far less than in the cells of the rib-meristem below. Difference in histological differentiation between the apex proper and the leaf primordium seems very minute at this stage.

The cells of the apical meristem of the dormant embryo show strong shrinkage of protoplasm under the technical procedures employed in this study.

6-hour shoot apex (Fig. 4B): The 6-hour shoot apex is nearly the same as that of the dormant embryo, except that leaf primordia have swollen up a little by hydration. The cells contain many aleurone grains, and show intense shrinkage of protoplasm. The cells still appear to remain inactive at this stage.

9-hour shoot apex: The cells of the apical meristem do no longer show shrinkage of protoplasm. In the cells of the leaf primordium, the cytoplasm stains deeply, and small-sized aleurone grains appear in place of larger ones. On the other hand, the cells of the shoot apex have digested most of aleurone grains.

The cells of the apical meristem seem to have become active physiologically by this time.

12-hour shoot apex (Fig. 4C): Aleurone grains have almost disappeared from the cells of the leaf primordium and the shoot apex, while the cells of the rib-meristem still contain plenty of them. All the cells stain intensely and appear to be fully activated.

15-hour shoot apex (Fig. 4D): Rising of the leaf primordium gets more conspicuous, and its cells stain deeper than those of the shoot apex. Cell divisions occur for the first time at this stage in the leaf primordium, but not yet in the shoot apex proper. The leaf primordium now begins to grow.

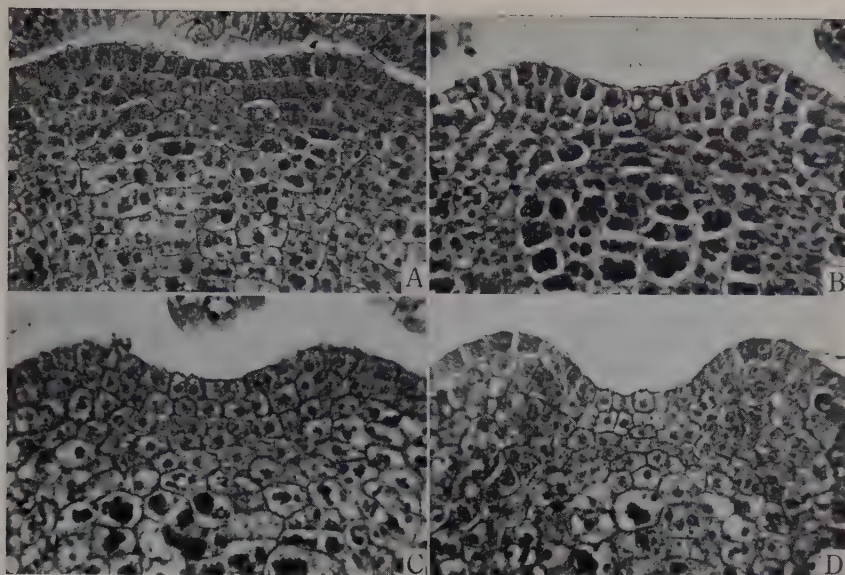


Fig. 4. Longitudinal sections of the shoot apices of germinating embryos. A, B, C and D, apices at the stages of 0, 6, 12 and 15 hours after sowing, respectively. A, $\times 210$; B and C, $\times 245$; D, $\times 225$.

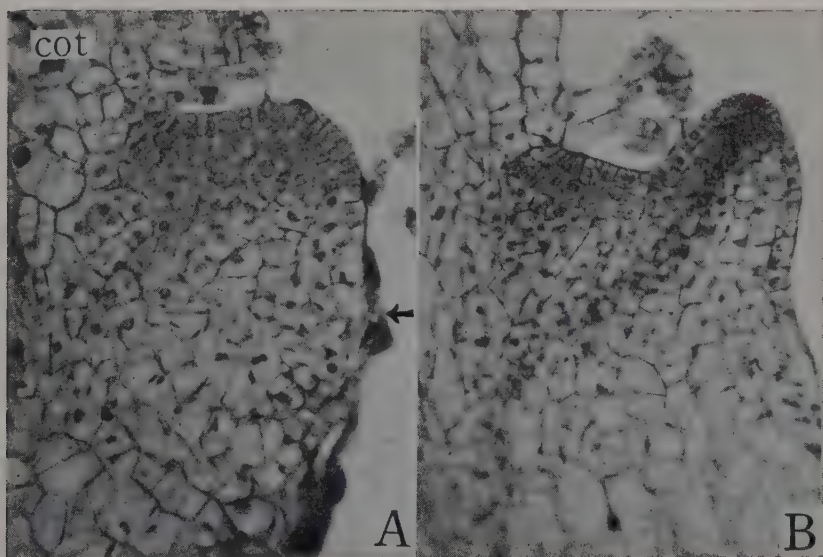


Fig. 5. Sections of regenerated shoot apices after the operation of the dormant shoot apex. A and B, 3 and 4 days after the operation, respectively. Note the epidermis, formed on the wounded side of the apex, extending down to the level of the arrow. cot: cotyledon. $\times 230$.

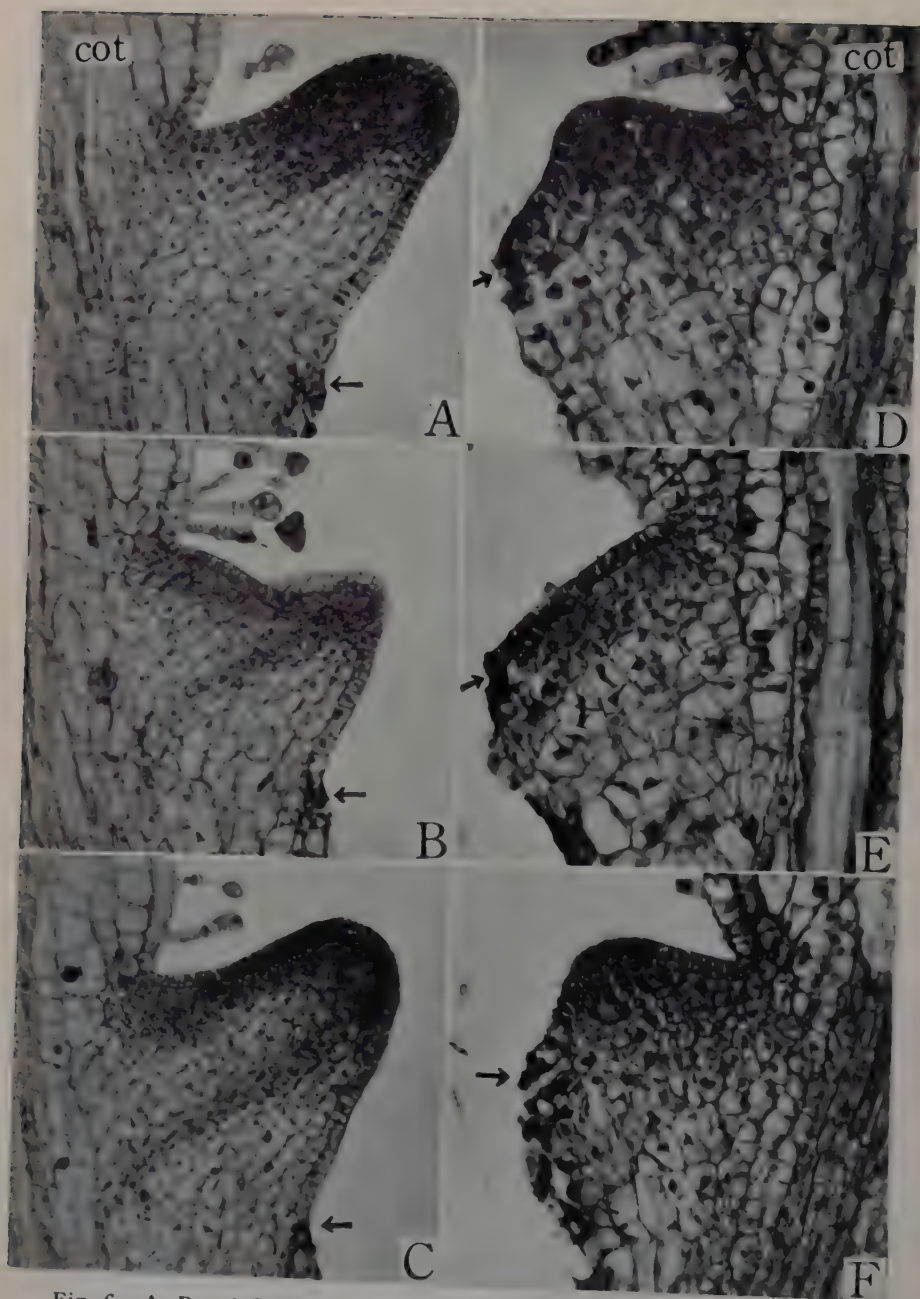


Fig. 6. A, B and C, three sections from a regenerated apex, 4 days after the operation of the dormant shoot apex. A and C, the side sections, and B, the median section of the apex. $\times 170$. D, E and F, three sections from a regenerated apex, 2 days after the operation of the 1-day-grown shoot apex. D and F, the side sections, and E, the median section of the apex. $\times 210$. Arrows indicate the lowermost end of the epidermis formed on the wounded side. cot: cotyledon.

24-hour shoot apex: Cell divisions take place in the shoot apex proper as well as in the leaf primordium, but more frequently in the latter. Staining of the cells of the leaf primordium is deeper than those of the shoot apex proper.

Within 24 hours after sowing, whole dimension of the shoot apex slightly increases mainly by cell enlargement rather than by the increment of cell number. But the relatively large rising of the leaf primordium is due not only to cell enlargement but also to cell increment.

From above observations, it may be said that the cells of the shoot apex of the embryo remain dormant till 6 hours after sowing, then become gradually active, but real growth occurs first in the leaf primordium 15 hours after sowing and then in the shoot apex proper.

By comparing these changes in the shoot apex with the changes in occurrence rate of double-leaf, it seems possible to draw a conclusion that the operation on the shoot apex can cause the double-leaf formation when the shoot apex stays dormant but the effect by the operation gradually decreases as the shoot apex gets metabolically active even before the beginning of growth, and finally it becomes lost after beginning of growth of the shoot apex.

3. *Regeneration of the shoot apex.*

Whether the first-leaf primordia develop as a double-leaf or as opposite leaves after the operation must be determined in the course of shoot regeneration. Therefore, some difference is expected to be found between the regeneration process of the dormant shoot apex and that of the growing shoot. In fact, a significant difference was found between these two processes.

It has been observed that the new shoot apex regenerated only from the uninjured surface of the incised apex, but never from the wounded surface, and that the wounded surface of the apical meristem showed mere wound responses, such as cell divisions parallel to the wounded surface or callus formation on the wound⁹⁻¹⁵). These results also apply to the present case, except that callus was not formed on the wound.

But a remarkable fact observed in the present study is the formation of new epidermis on the wounded side of the incised apex. As will be described below, it is significant that the new epidermis could be observed in the shoot apex bearing a double-leaf or a single-leaf, but not in the shoot apex bearing opposite leaves.

Fig. 5A shows a regenerating shoot apex 3 days after the operation of a dormant shoot apex. New shoot organization appears nearly completed. In the figure, the first leaf primordium is expected to arise on the right side of the new apex. What is worth mentioning here is the fact that a distinct epidermis covers the wounded side of the apex down to the level indicated by an arrow. This level of the lowermost end of the epidermis is considerably lower than that of the summit of the shoot, while at this stage the shoot apex itself shows no growth in height at all. Therefore, the epidermis on the wounded side must have been reorganized from the underlying cells of the wound, or formed from the tunica layer by its downward elongation along with the elongation of the subjacent tissue. Remains of dead cells attaching on the epidermis may support the former possibility. The lowermost part, at least, of the epidermis looks to be formed in this way. But the regeneration of organized tissue from the wounded surface of the apical meristem seems to be very difficult, and, in fact, it has never been reported. Although Ball¹³) stated that epidermis was formed from the sub-wound cells of the pith plug isolated from the sur-

rounding tissue of the shoot apex in *Lupinus*, the epidermis does not look to be typically organized one and the real epidermis seems to be formed as a result of growth of the regenerated apex. Thus, in the present study, the larger portion of the new epidermis was perhaps formed by downward extension of the tunica.

The downward extension of the tunica together with the elongation of the sub-jacent tissue causes a strong distortion in the outline of the shoot apex. The distortion of the external shape should be attended by the changes in the internal structure such as histological zonation or structural pattern in the shoot apex. Thus the fact of the epidermis formation on the wounded side of the incised apex suggests that profound disorganization and subsequent reorganization of the apical structure have occurred in the course of shoot regeneration.

Fig. 5B shows a regenerated shoot apex, 4 days after the operation, in which the epidermis has extended downward on the wounded side and a leaf primordium has started to grow. In comparison with the growth of normal first-leaf primordium, which attains a height of 600 to 700 microns and commences laminal development 4 days after sowing, that of the operated leaf primordium is very much retarded, because it takes about 3 days for reorganization of the new shoot apex. Examination of serial sections shows that this apex bears only one leaf primordium initiated opposite to the cotyledon.

A, B and C in Fig. 6 represent three sections taken from an apex bearing a double-leaf primordium, 4 days after the operation of a dormant embryo. A and C are both the side sections, and B the median section of the shoot apex. It can be seen in the B section that the basal parts of the primordia A and C are connected, that is, these two primordia are to grow into a double-leaf. In this apex the epidermis on the wounded side extends down to the level obviously lower than the summit of the shoot.

In all examples above mentioned, it is plain that the shoot apex itself has not yet grown in height at all, because it stays at the same level as in the dormant embryo, i. e., at the base of the cotyledons. Therefore it is doubtless that the epidermis extended downwards from the shoot summit.

In contrast with above examples, in the following example the operation was made 24 hours after sowing. D, E and F in Fig. 6 are the sections from the shoot apex 2 days after the operation. Both D and F represent the side sections, and E the median section of the shoot apex. On this apex two opposite leaf primordia developed. In this case, in contrast with above cases, the wounded side completely lacks epidermis up to the uppermost level. The wounded surface is covered only by irregular cell layer, not by an organized epidermis. This means that the tunica did not extend downwards, but extended only outwards enlarging the area of the apex. Accordingly it may be said that the apex did not undergo strong distortion in the course of regeneration. The development of this apex is much more advanced than that of the apex which formed new epidermis on the wounded side after the same growth period (cf. Fig. 5A). It is only one day retarded as compared with the intact apex. Therefore it is probable that the original organization of the apex was not so much disturbed by the operation, and the shoot apex regeneration took place readily by mere repairing or expansion, rather than by reorganization, of the halved apical meristem.

Thus the formation of organized epidermis on the wounded side of the incised apex is the characteristic of the shoot regeneration in the course of which leaves of D-group are formed. In other words, the disturbance of the apical organization, the epidermis formation on the wounded side of the apex during its reorganization, and

the formation of D-group leaves, are perhaps the closely connected and consecutive events.

4. Interpretation of the process of the double-leaf formation.

Previously the author⁷⁾ presented an explanation of the process of the double-leaf formation. That, however, referred mainly to the approaching of two opposite leaves. Although the approaching of the leaf primordia must be one of the important conditions for the double-leaf formation, there still seems to remain some gap to be explained between a mere fact of spatial approaching of the leaf primordia and their fusion into one.

In relation to this point, interpretations of gamophyll formation induced by means of growth substance application give a good suggestion for the explanation of the double-leaf formation.

Plantefol^{16,17)} investigated gamophyll induction by 2,4-D in *Linum usitatissimum*. After him, gamophyll formation depends on abnormal functioning due to 2,4-D of whole leaf-generating region ("initial ring"). That is, the simultaneous development of the whole initial ring results in the formation of a tubular union of leaves. Haccius and Schneider¹⁸⁾ presented similar explanation in *Galium aparine*. They concluded that the morphogenetic pattern in the apical cone based upon some gradients of substances exuding from definite poles was flattened by excess amount of 2,4-D, and consequently separation of individual leaf primordia within the leaf generating region did not take place and the entire "Bildungsring" grew into a tubular structure (gamophyll).

Consulting these interpretations on gamophyll induction, the double-leaf formation may be explained as follows.

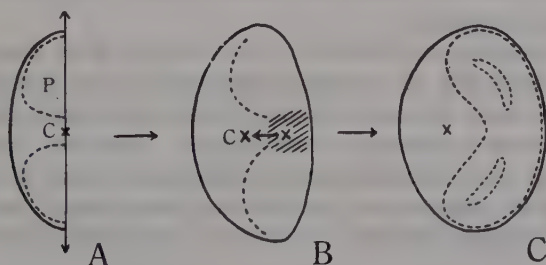


Fig. 7. Schematic representation of the process of fusion of two opposite leaf primordia. C: apex center. P: leaf primordium. Further explanation in the text.

When the shoot apex of dormant embryo is split (Fig. 7A), the apex may undergo profound disorganization, as suggested in the preceding section. Bisected leaf primordia may also degenerate to various extent. Upon regeneration, the new apex center shifts toward outside (Fig. 7B), and the original central region (hatched area in Fig. 7B) comes to occupy the lateral region of the new apex. Then this region may become potential for leaf formation. In consequence, there occurs on one side of the apex a wide leaf forming area consisting of both the newly activated region and the degenerated leaf primordia. When the leaf primordia resume growth after some retardation period, the region between the primordia may grow with them. Thus two primordia may grow into a united structure (Fig. 7C). On that occasion, the narrow shoot apex of sesame embryo and accordingly small distance between two

leaf primordia may be one of the conditions which facilitate the process. Grade of disorganization of the shoot apex and the size of the regenerated apex may determine the degree of fusion. If the disorganization is strong and the regenerated apex is narrow, the leaf forming area may be small, and a single leaf may be formed.

On the other hand, the active shoot apex may undergo little disturbance in its structure when operated, and its original configuration may persist. Regeneration of the shoot apex may be performed by mere expansion of the halved apex. Therefore the leaf primordia may maintain their individualities and their growth may not be so much inhibited. They may only perform regeneration of their own structure, growing separately, although they may approach to each other more or less as a result of outward shift of the new apex center.

Summary

1) Double-leaves were formed in high frequency in *Sesamum indicum* by splitting the shoot apex of dormant embryo. But their occurrence rate decreased rapidly with delaying the operation stages from 6 to 15 hours after sowing, and later than 15 hours after sowing they were scarcely formed, opposite leaves being formed in place of them.

2) Histological observation revealed that the shoot apex of embryo stayed dormant till 6 hours after sowing, then became gradually activated metabolically, and that cell division occurred first 15 hours after sowing. Therefore plastochronic change hardly occurred by that time.

3) From above results, it may be concluded that the double-leaf formation is the characteristic response of the dormant shoot apex to the operation, and the response becomes different as the shoot apex gets active, even if its plastochronic stage is the same as in the dormant stage.

4) When the operation was made on the dormant shoot apex, an epidermis was formed on the wounded side of the apical meristem of the regenerated shoot, while it was never formed after the operation on the activated shoot apex. The epidermis was formed by downward extension of the tunica. This fact means that the apical meristem, when split at the dormant stage, undergoes strong distortion in the course of regeneration. The distortion in the external shape may be attended with the disturbance in the internal structure. This is a characteristic behavior of the dormant shoot apex.

5) Process of the double-leaf formation was interpreted from the viewpoint of disturbance in the structure and morphogenetic function of the shoot apex due to the operation.

References

- 1) Fujita, T., Plant Teratology (in Japanese), Tokyo (1949).
- 2) Snow, M., and Snow, R., Phyl. Trans. Roy. Soc. London B **222**: 353 (1933).
- 3) —, and —, *ibid.* **225**: 63 (1935).
- 4) —, and —, New Phytol. **36**: 1 (1937).
- 5) Snow, R., *ibid.* **41**: 108 (1942).
- 6) Hanawa, J., and Ishizaki, M., Sci. Rep. Fac. Lib. Arts and Educ. Gifu Univ. **1**: 55 (1953).
- 7) Hanawa, J., Bot. Mag. Tokyo **72**: 425 (1959).
- 8) —, *ibid.* **70**: 203 (1957).
- 9) Pilkington, M., New Phytol. **28**: 37 (1929).
- 10) Ball, E., Symp. Soc. Exp. Biol. II: 246 (1948).
- 11) —, Amer. Jour. Bot. **37**: 117 (1950).
- 12) —, Science **112**: 16 (1950).
- 13) —, Amer. Jour. Bot. **39**: 167 (1952).
- 14) —, *ibid.* **42**: 509 (1955).
- 15) Soma, K., Jour. Fac. Sci. Univ. Tokyo Sect. III, Bot. VII: 199 (1958).
- 16) Plantefol, L., Compt. Rend. Acad. Sci. Paris

235: 386 (1952). 17) —, *ibid.* 235: 812 (1952). 18) Haccius, B., and Schneider, W., *Planta* 52: 206 (1958).

摘 要

埴 順: ゴマの双生葉形成に関する研究・続報

1. ゴマの胚の生長点を、対生する第1葉原基を通る面で二分すると、第1葉原基はしばしば双生葉となる。手術の時期をいろいろに変えてみると、双生葉は、手術が休眠状態の胚の生長点になされたときに形成されるが、胚が発芽活動にはいると、まだ生長は起こらなくても双生葉形成率は急に減り、細胞分裂が始まるころにはほとんどそれは形成されなくなることが知られた。

2. 休眠胚の生長点の手術ののちには、再生した生長点の、傷をうけた側にあらたに表皮が生じた。24時間胚の生長点ではこのことは起こらない。新しい表皮は *tunica* が下方に向かって引き伸ばされることによって形成される。したがって、この場合には生長点に強い歪曲が起こったことになる。生長点の内部構造にも強い乱れが起こっているであろう。

3. 双生葉形成過程は、手術による生長点の構造および機能の乱れ、ならびにその後の再編成ということから説明された。(東京都立大学理学部生物学教室)

The Effects of Temperature after the Light-exposure on the Germination of *Oenothera* Seeds

by Tadashi FUJII* and Sigeo ISIKAWA*

Received June 13, 1961

The interaction between photoperiod and temperature in seed germination has been investigated by many workers¹⁻⁴). The results obtained have indicated the diversity in the patterns of the interaction in different kinds of seeds.

In 1955, Black and Wareing¹) demonstrated in the seeds of *Betula pubescens* that the effect of increased temperature appeared to be primarily upon certain processes initiated by a preceding period of illumination. On the other hand, Isikawa and Ishikawa³), and Fujii and Isikawa⁴), dealing with the interesting mode of germination, reported that the seeds of *Elsholtzia* and *Nasturtium* required for their full germination a certain period of a low temperature (5°) applied immediately after the light irradiation. They⁴) also demonstrated that their germination was suppressed when a dark period of high temperature (23°) was inserted between the light irradiation and the low-temperature treatment, and this inhibitory effect of darkness at high temperature was removed effectively by the "light-break." The results indicated that the changes during the dark periods of high temperature in *Nasturtium* seed germination resembled those in the flowering of long-day plants.

The authors obtained results similar to those on *Nasturtium* seeds concerning the germination of *Oenothera Lamarckiana* Ser. seeds which have been regarded as being typical light-sensitive seeds⁵). The present study aims at analysing the role of temperature in the germination of light-sensitive seeds.

Material and Methods

In these studies seeds of *Oenothera Lamarckiana* Ser. were used. A collection of seeds was made in October 1960 at Fujiyoshida, Yamanashi Prefecture.

The methods used were similar to those previously described for *Nasturtium* seeds by Fujii and Isikawa⁴). In testing the effect of a particular treatment, seeds were placed on 0.7-0.8% agar-beds in Petri-dishes. Two lots of 100 seeds were used for each treatment. Each dish was wrapped with a thick black paper and placed in an incubator which was usually controlled within $\pm 1^\circ$ during the dark period, and the seeds were treated in thermostatically controlled light cabinets during the light period.

Main light periods were generally maintained with cool white fluorescent light, the intensity being measured by a photometer at the surface of Petri-dishes. The red radiation was given from cool white fluorescent lamps through a filter of two layers of red cellophane.

The seeds with grown radicles on the third day after the treatment were counted as those germinated. The average germination rate of seeds in two dishes being subjected to the same treatment was taken as the germination rate of the lot. The experiments reported here were performed during the period from February to April, 1961.

* Botanical Institute, Faculty of Science, Tokyo University of Education, Otsuka, Tokyo, Japan.

Results

In preliminary tests, it is indicated that the seeds of *Oenothera Lamarckiana* Ser. will not germinate in complete darkness at various temperatures, but in continuous illumination at 2000 lux from the standard cool white fluorescent lamps. They are thus light-requiring seeds.

To observe the sensitivity to light, the seeds were irradiated for 6 hrs. with the light of 2000 lux after various times of dark imbibition, and then kept in darkness until no further germination took place (about 3 days). Data not given in the figure showed that there was a gradual increase in sensitivity to light with time, the maximum percentages of germination being attained after 2 days of imbibition at 35°, 3 days at 30°, and 3.5 days at 23°, and then decreased gradually with further increase in the imbibition time.

The following experiments were then carried out to determine the effect on germination of exposing the seeds to irradiation of various lengths. The seeds to be exposed to light at 35°, 30° and 23° were soaked in darkness for 2, 3, and 3.5 days, respectively. The results are shown in Fig. 1. It was seen that, at 23°, exposure to a short illumination, even of 6 hours' duration, results in a considerably high percentage of germination. A rise in temperature to 30° or 35°, on the other hand, decreased the response to the light irradiation, in the case of short applications. This decrease of germination percentage, however, was eliminated markedly with the prolonged exposure to light (Fig. 1). The results indicated that the processes inhibited with high temperature application were involved in germination, this inhibitory effect being removed by the longer application of illumination.

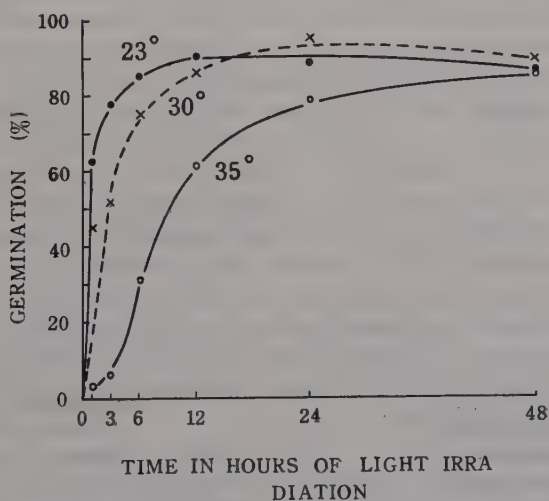


Fig. 1. Effect on germination of irradiation (with 2000 lux light) of various durations.

From the results of the preceding experiments, it is not possible, however, to analyze the processes which are primarily affected by an increase in temperature. The experiment was performed to observe whether it was the light reactions or the dark reactions which were inhibited by high temperature. The seeds to be exposed for 6 hours to the light of 2000 lux were soaked in the dark for 72 hrs. at 30° before illumination, and then kept in darkness for 3 days after the irradiation. During the

Table I. Effect of temperatures during the light and dark periods.

72-hr. dark imbibition before irradiation	6-hr. light period	Dark period after irradiation	Germ. %
30°	35°	35°	37.0
	35°	30°	72.5
	30°	35°	29.0
	30°	30°	74.0

light period and the following dark period, the seeds were exposed to temperatures of 30° and 35°, as shown in Table I.

The germination percentages were about 30% in seeds kept at the higher temperature (35°) after the light exposure and more than 70% in seeds kept at 30° after that. These results indicate that the temperature during the dark period given after the light exposure is primarily effective in stimulating or inhibiting germination.

An attempt was made to obtain more information of this inhibitory effect of higher temperature (35°) by varying the length of temperature treatment after the light exposure. *Oenothera* seeds in several dishes were irradiated for 6 hrs. at 30° with the light of 2000 lux after 3 days of dark imbibition at the same temperature, and each dish of seeds was transferred in the dark to the cabinet at 35°. After this temperature treatment for various durations, they were returned to the cabinet at 30° in the dark. In this experiment, as the duration of high temperature after the light irradiation was increased, the germination percentages gradually decreased to about 30% as shown in Fig. 2.

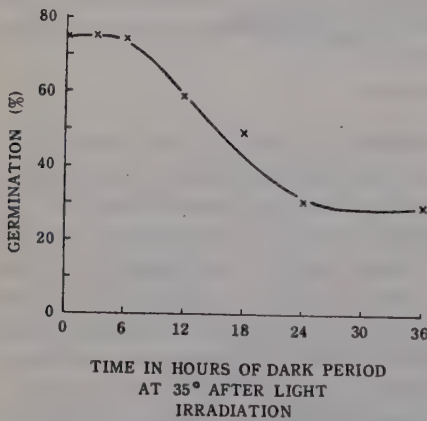


Fig. 2. Effect of dark incubation at 35° after 6-hr. light period.

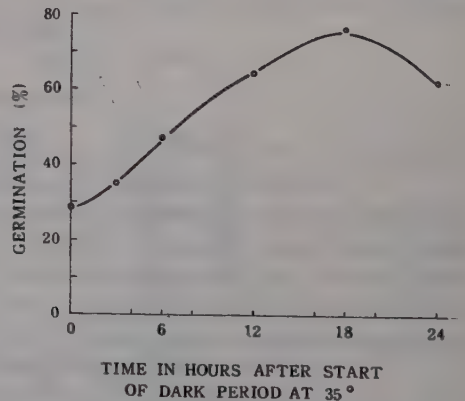


Fig. 3. Effect on germination of a 10-min. light-break given at various times in the 24-hr. dark period at 35° after illumination.

It has been reported that germination response in *Nasturtium* seeds is markedly modified if the dark period at 23° between the light and the low temperature period is interrupted with a short period of illumination⁴). This inhibitory effect of dark period might be compared with that of high temperature in the present experiment. It is therefore of considerable interest to investigate the effect of "light-break" during the dark period of higher temperature, on the germination of *Oenothera* seeds.

The seeds were first irradiated for 6 hrs. with the light of 2000 lux from the standard cool white fluorescent lamps after 3 days of dark imbibition at 30°, and immediately exposed to higher temperature (35°) in darkness for 24 hrs. A 10-minute light-break with red light was given at some point during the dark period at 35°. The light-break was most effective when given at about the 3/4 point of the dark period of higher temperature (Fig. 3). The results obtained showed that the germination of *Oenothera* seeds was affected by light-break, similarly to the case of the germination of *Nasturtium*⁴⁾ seeds and the flowering of long-day plants.

Discussion

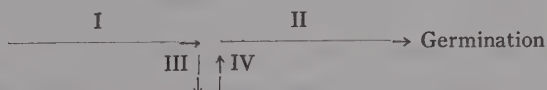
Black and Wareing¹⁾ observed that a higher temperature (20°) in the dark storage period following a single photoperiod served to increase the rate of germination. They also demonstrated that high temperature during the dark period after the light-exposure was primarily effective in stimulating germination, but high temperature during the light period was not. Isikawa and Ishikawa³⁾, on the other hand, observed that, other than illumination, a low temperature treatment immediately after the light irradiation was necessary for inducing the germination of a species of *Elsholtzia*.

The foregoing experiments in this paper provide a clear evidence that the high temperature (35°) after the irradiation is inhibitive on the germination of *Oenothera* seeds which are known as typical light-sensitive seeds. From the results in *Betula*, *Nasturtium* and *Oenothera*, it may be considered that the moderate temperature for germination is represented by the temperature immediately after the light-exposure, in light sensitive seeds except the seeds of some species of *Lepidium*⁶⁾, *Rumex*⁷⁾ etc. only a single short exposure to light is sufficient to bring about germination.

The photoperiodic control of seed germination has been reported in a number of photoblastic seeds^{1,2,4,8,9)}. One of the characteristic features associated with the photoperiodic control of germination is that the germination response is affected by the length of the dark period, and that this response is markedly modified if the dark period is interrupted with a short period of illumination.

In *Oenothera* seeds, a dark incubation at 35° after the light irradiation is inhibitory to the germination. The germination percentage, however, is increased if the dark period of 35° is interrupted with a short period of irradiation. Thus, the processes occurring during the high-temperature treatment after the light-exposure resemble those during the dark period in the flowering of long-day plants.

Namely, the sequence of the processes in *Oenothera* seeds can be illustrated, in analogy to that reported in *Nasturtium* seeds⁴⁾, by the following figure:



I a light-dependent process,

II a moderate temperature-requiring process,

III a degradative process with high temperature,

IV a low-intensity light process antagonistic to the degradative process.

The seeds require a moderate temperature after the light-exposure for their germination. If they are treated with high temperature, the high temperature after the irradiation tends to destroy the changes which have taken place in the preceding light period, but if the light period is extended beyond a certain limit at 35°, the

germination is markedly promoted. It may be considered that this promotion of germination is due to the inhibition of degradative process of high temperature with the light irradiation which is regarded as being a low-intensity light (light-break).

Summary

1. The seeds of *Oenothera Lamarckiana* Ser. do not germinate in darkness at various temperatures, but exposure to a short illumination at a temperature of 23°, results in a considerably high percentage of germination. Thus, the seeds of *Oenothera* are shown to be light-requiring for their germination.

2. The temperature in the dark, but not in the light is effective in stimulating or inhibiting germination. When the seeds are exposed to higher temperature (35°) immediately after the light irradiation, their germination is suppressed. This inhibitory effect is removed if the dark period of 35° is interrupted with a short period of illumination (light-break). Thus, the processes occurring during the dark period of 35° after the light irradiation resemble those in the flowering of long-day plants.

3. The observed results in this paper suggest that the inhibitory effect of dark period on germination of *Oenothera* seeds is virtually the degradative action of high temperature on the processes initiated by preceding period of illumination.

References

- 1) Black, M., and Wareing, P. F., *Physiol. Plantarum* **8**: 300 (1955).
- 2) — and —, *Jour. Exp. Bot.*, **11**: 28 (1960).
- 3) Isikawa, S., and Ishikawa, T., *Plant and Cell Physiol.* **1**: 143 (1960).
- 4) Fujii, T., and Isikawa, S., *ibid.* **2**: 77 (1961).
- 5) Isikawa, S., and Shimogawara, G., *J. Japan Forestry Soc.* **36**: 318 (1954).
- 6) Toole, E. H., Toole, V. K., Borthwick, H. A., and Hendricks, S. B., *Plant Physiol.* **30**: 473 (1955).
- 7) Isikawa, S., and Fujii, T., *Plant and Cell Physiol.* **2**: 51 (1961).
- 8) Vaartaja, O., *Canad. Jour. Bot.* **34**: 377 (1956).
- 9) Isikawa, S., *Bot. Mag. Tokyo* **67**: 51 (1954).

摘 要

藤伊正, 石川茂雄: オオマツヨイグサ種子の発芽に対する光照射後の
温度の影響について

従来, 典型的な光発芽種子として知られていたオオマツヨイグサの種子は, その発芽過程において光照射直後の反応に狭温性を示し, 6 時間の光照射後 23~30° の温度におかれた場合には高い, 発芽率を示すにもかかわらず, 低温 (15°) あるいは高温 (35°) におかれた場合には, その発芽率はいちじるしく抑制されることが観察された。

このことは, この種子の発芽における“適温”が光照射後の過程に対する適温であることを暗示している。

この報告では高温におかれた場合の抑制についてのみ取り扱ったが, 著者がさきに報告したスカシタゴボウの種子の発芽や花芽形成における長日植物に見られる光中断と同様に光照射後の 35° の暗期に赤い光を短時間照射することによって, この高温の抑制効果のとりぞかれることが見いだされた。

この種子の発芽における“暗期”の抑制的効果は, 実は光照射後の反応に対する“高温”の抑制的効果に基づくものであることを暗示した。(東京教育大学理学部植物学教室)

交配からみたシメジの分類学的考察

広 本 一 由*

Kazuyoshi HIROMOTO: A Discussion on the Taxonomy of *Tricholoma aggregatum* (Schaeffer ex Secretan) Constantin et Dufour and *Tricholoma conglobatum* (Vittadini) Saccardo

—From the Viewpoint of Crossing—

1961 年 4 月 28 日受付

シメジは多型性である関係上、多数の俗名が与えられ、学名においても異種同名あるいは同種異名がみられ、従来、分類学上の混乱をきたしている。そこで、今関¹⁾はこの混乱を除くために、日本産シメジの分類学的再検討を試み、主として形態的特徴より、これを大黒系と千本系の2系に大別し、前者はホンシメジ(一名ダイコクシメジ) *Tricholoma aggregatum* (Schaeff. ex Secr.) Const. et Duf., 後者はシャカシメジ(一名センボンシメジ) *Tricholoma conglobatum* (Vitt.) Sacc. のそれぞれ一種に統一するのが妥当であるとした。著者は 1954 年 12 月 12 所の林地において、大黒系および千本系シメジの生態・形態などを観察するとともに、担子胞子の発芽、菌の培養などについて実験を行ない、さらに最近、形態を異にする大黒系シメジ相互の間および大黒系シメジと千本系シメジの間において交配実験を行なったところ、形態上からシメジを前述の二系に分類することを妥当とした今関の説とまったく一致する結果をえたので、ここに報告する。

実験材料

大黒系シメジの発生地 9 カ所において、6 カ年間(1954~1959)にわたり、子実体の発生状態・形態などを観察した結果、形態的に 4 型を区別すること

ができたので、以下、これらをホンシメジ A, B, C および D としてあらわす。このうち A, C および D はすでに、それぞれ *Tricholoma aggregatum*, *Agaricus amplus* および *A. decastes* の学名が与えられているものと同一物と思われる。また B の特徴を示す記載については、まだみたことがない。シャカシメジについては、広島県向原町、長野県大桑村および福島県都路村の 3 カ所において調査したが、発生地による形態的相違はみられなかった。本実験に用いた、大黒系および千本系シメジの特徴および調査地を第 1 表に掲げた。

実験方法

1. 培養基

担子胞子の発芽用培養基と、菌糸の培養または交配用の培養基とは組成の異なるものを用いた。

1) 担子胞子の発芽用培養基としては、エビオス煎汁(乾燥ビール酵母)にジャガイモ煎汁をくわえた寒天培養基を用いた。その組成は、ジャガイモ煎汁 95 ml., エビオス煎汁(1%) 5 ml., しょ糖 2 g. および寒天 2 g. である。

2) 菌糸の培養および交配用培養基としては、エビオス煎汁、ジャガイモ煎汁に松葉煎汁をくわえた寒天培養基を用いた。その組成は、松葉煎汁²⁾ 40 ml., エビオス煎汁(1%) 20 ml., ジャガイモ煎汁 20 ml., しょ糖 2.4 g., 寒天 2.4 g., 水 40 ml., および 1 規定苛性ソーダ 0.1~0.2 ml. (pH 5.5~6.0) である。

* 山口県熊毛南高等学校 Kumage Minami High School, Yamaguchi Prefecture, Japan.

Table 1. シメジの名称, 調査地およびその特徴

名 称	調 査 地	子実体の主な形態的特徴
ホンシメジA <i>Tricholoma aggregatum</i>	柳井市伊陸 同日積 山口県由宇町寺迫	多数の子実体が一塊となって発生する。菌柄下部が膨大し、大黒系シメジの典型的な形態を示す。
ホンシメジB	広島県向原町	子実体の形態はAに同じ。子実体の他に鶏卵大の白色菌糸塊を生ずる。この菌糸塊からは、ぎわめてまれに1~2本の小形子実体を生じることがある。
ホンシメジC <i>Agaricus amplus</i>	山口県周東町 柳井市伊陸久賀地 長野県大桑村	大形で孤生することが多い。菌柄は菌蕾のときから上下同大。非常にまれに菌傘表面から新らしく小形子実体を数本生じることがある。
ホンシメジD <i>A. decastes</i>	山口県由宇町笠塚 柳井市伊陸木部	一塊をなして多数叢生するが、菌柄が比較的長く、上下同大である。
シャカシメジ <i>Tricholoma conglobatum</i>	広島県向原町 長野県大桑村 福島県都路村	塊茎状の共通の株から小形の子実体が多数叢生する。菌柄は細くて上下同大。秋季、ホンシメジよりも約10日間早く発生する。

2. 担子胞子の播種

Fig. 1 に示すような播種装置を用いた。すなわち、孢子発芽用の培養基をガラス槽内に入れ、ガラ

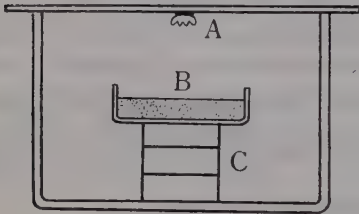


Fig. 1. 担子胞子播種装置

A: 菌傘の一部。B: 培地。

C: 培地面の高さを調節する支台

スふたの中央に菌傘の一部をワセリンではりつけ、菌傘と培養基面との間を2~6 cm.の間隔に保ち、落下する孢子が空中においてある程度分散したのちに培養基面に達するように工夫した。この播種法をかりに空中懸濁播種法と呼ぶ。この播種法は、a) 孢子の播種密度は菌傘の直下が最大で、その周辺に行くにつれて連続的に小になるから、疎部から発芽胞子を探したすのに時間がかからず、単胞子培養に便利である。b) 菌傘と培養基面の間隔を適宜加減すると、孢子の空中における分散度と落下時間が加減できるので、一定の孢子密度を得るに必要な播種時間を適当に調節することができるなどの特徴を有する。

3. 担子胞子の単胞子培養

空中懸濁播種法により、孢子発芽用の培養基に胞子を播種し、27°に保つと、4~5日後には発芽を開始するから、そのうち生育良好と思われる単胞子を拾いとり、菌糸培養基に移した。菌糸そうがよく発育した後、再三かすがい連結の有無を検し、10個以上の単胞子培養を得て交配実験に供した。

4. 単胞子培養相互間の交配

単胞子培養のそれぞれに番号を付し、交配用培養基上に約5 mm.の距離をおいて、2~3 mm.²の移植片を相対峙させておのおのを組み合わせ、各組み合わせとも、ペトリ皿3個について実験を行なった。温度を25°に保つと、10日後には両菌糸そうがたがいに交錯するから、その部分の菌糸についてかすがい連結の有無を検した。

実験結果と考察

ホンシメジの4型およびシャカシメジの単胞子培養の菌糸、すなわち一次菌糸はかすがい連結を形成しないが、二次菌糸はこれを形成する。子実体を構成している菌糸および子実体から分離した菌糸には、常にかすがい連結がみられること、子実体から分離した菌糸と交配によって得た菌糸とはまったく同一であることなどの事実から、おそらく本菌はヘテロタリクスの菌であろう。

Tables 2~10 の因子記号は、西門・木村⁹⁾、河村⁹⁾、伊藤⁹⁾らにならって、かりに付したものである。本実験においては細胞学的観察は行なわなかつ

たが、単孢子培養のかすがい連結のない菌糸は一核菌糸、交配菌糸でこれを有するものは二核菌糸と考えられる。両菌糸はかすがい連結の有無で容易に区別されるが、その他の点においては外観上の差異は認められない。

1) ホンシメジ A, B, C および D の極性

ホンシメジ A, B, C および D のそれぞれにおいて、1 個の子実体から得た単孢子培養についてすべての組合わせて交配を行なった結果を、Tables 2~5 に掲げた。すなわちこれらはいずれも 4 極性である。

Table 2. All possible pairings of 14 monosporous mycelia isolated from a single fruit body of the type A of *Tricholoma aggregatum*.

	\overbrace{AB} 5, 7, 8, 13, 14	\overbrace{ab} 6, 9	\overbrace{Ab} 1, 2, 4, 11, 12	\overbrace{aB} 3, 10
$AB \begin{Bmatrix} 5 \\ 7 \\ 8 \\ 13 \\ 14 \end{Bmatrix}$	-	+	-	-
$ab \begin{Bmatrix} 6 \\ 9 \end{Bmatrix}$	+	-	-	-
$Ab \begin{Bmatrix} 1 \\ 2 \\ 4 \\ 11 \\ 12 \end{Bmatrix}$	-	-	-	+
$aB \begin{Bmatrix} 3 \\ 10 \end{Bmatrix}$	-	-	+	-

Table 3. All possible pairings of 10 monosporous mycelia isolated from a single fruit body of the type B of *Tricholoma aggregatum*.

	\overbrace{AB} 5, 6	\overbrace{ab} 1, 3, 7	\overbrace{Ab} 4, 9, 10	\overbrace{aB} 2, 8
$AB \begin{Bmatrix} 5 \\ 6 \end{Bmatrix}$	-	+	-	-
$ab \begin{Bmatrix} 1 \\ 3 \\ 7 \end{Bmatrix}$	+	-	-	-
$Ab \begin{Bmatrix} 4 \\ 9 \\ 10 \end{Bmatrix}$	-	-	-	+
$aB \begin{Bmatrix} 2 \\ 8 \end{Bmatrix}$	-	-	+	-

Table 4. All possible pairings of 14 monosporous mycelia isolated from a single fruit body of the type C of *Tricholoma aggregatum*.

	\overbrace{AB} 1, 2, 8, 9, 11	\overbrace{ab} 3, 5, 6	\overbrace{Ab} 7, 10, 12, 13	\overbrace{aB} 4, 14
$AB \begin{Bmatrix} 1 \\ 2 \\ 8 \\ 9 \\ 11 \end{Bmatrix}$	-	+	-	-
$ab \begin{Bmatrix} 3 \\ 5 \\ 6 \end{Bmatrix}$	+	-	-	-
$Ab \begin{Bmatrix} 7 \\ 10 \\ 12 \\ 13 \end{Bmatrix}$	-	-	-	+
$aB \begin{Bmatrix} 4 \\ 14 \end{Bmatrix}$	-	-	+	-

Table 5. All possible pairings of 13 monosporous mycelia isolated from a single fruit body of the type D of *Tricholoma aggregatum*.

	\overbrace{AB} 3, 6, 7	\overbrace{ab} 1, 2, 5, 12	\overbrace{Ab} 4, 11, 13	\overbrace{aB} 8, 9, 10
$AB \begin{Bmatrix} 3 \\ 6 \\ 7 \end{Bmatrix}$	-	+	-	-
$ab \begin{Bmatrix} 1 \\ 2 \\ 5 \\ 12 \end{Bmatrix}$	+	-	-	-
$Ab \begin{Bmatrix} 4 \\ 11 \\ 13 \end{Bmatrix}$	-	-	-	+
$aB \begin{Bmatrix} 8 \\ 9 \\ 10 \end{Bmatrix}$	-	-	+	-

2) ジャカシメジの極性

Table 6 に示すとおりジャカシメジも 4 極性である。

3) ホンシメジ A と B, C および D との交配

ホンシメジ 4 型において、それぞれ極性の判明した一次菌糸を使って A を B, C および D のそれぞれと交配した結果を Tables 7~9 に掲げた。すなわ

Table 6. All possible pairings of 12 monosporous mycelia isolated from a single fruit body of *Tricholoma conglobatum*.

	A B		a b		A b		a B	
	103, 107	106	108, 110	109, 112	105, 111	101, 104	102	
A B { 103 106 107	-		+		-		-	
a b { 108 109 110 112	+		-		-		-	
A b { 105 111	-		-		-		+	
a B { 101 102 104	-		-		+		-	

Table 7. Result of pairing between tetrapolar haplonts of the type A and B in *Tricholoma aggregatum*.

		Type A			
Type B		A B 5	a b 6	A b 1	a B 3
	A B 5	+	+	+	+
	a b 1	+	+	+	+
	A b 4	+	+	+	+
	a B 2	+	+	+	+

Table 8. Result of pairing between tetrapolar haplonts of the type A and C in *Tricholoma aggregatum*.

		Type A			
Type C		A B 5	a b 6	A b 1	a B 3
	A B 1	+	+	+	+
	a b 3	+	+	+	+
	A b 6	+	+	+	+
	a B 4	+	+	+	+

ち、ホンシメジAとB、CおよびDの間ではいずれも癒合がおこる。したがって、これら4型は、形

Table 9. Result of pairing between tetrapolar haplonts of the type A and D in *Tricholoma aggregatum*.

		Type A			
Type D		A B 5	a b 6	A b 1	a B 3
	A B 3	+	+	+	+
	a b 1	+	+	+	+
	A b 4	+	+	+	+
	a B 8	+	+	+	+

態的にはかなりいちじるしい相違があるが、同種とみなすのが妥当である。

4) ホンシメジAとジャカシメジの交配

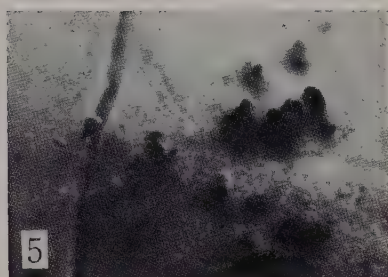
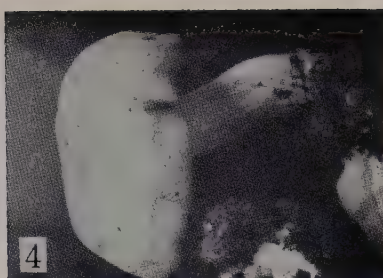
1) および 2) によって、極性の判明した単相菌系を使って、ホンシメジAと広島産ジャカシメジとの間において交配を行なった結果を Table 10 に掲げた。すなわち、両者間にはいずれも癒合がおこらないから、両者は種を異にするとみなすのが妥当である。

Table 10. Result of pairing between tetrapolar haplonts of the type A of *Tricholoma aggregatum* with tetrapolar haplonts of *Tricholoma conglobatum*.

		The type A of <i>T. aggregatum</i>			
<i>T. conglobatum</i>		A B 5	a b 6	A b 1	a B 3
	A B 103	-	-	-	-
	a b 108	-	-	-	-
	A b 105	-	-	-	-
	a B 101	-	-	-	-

摘 要

1) ホンシメジの発生林地9カ所を調査した結果、発生地が異なると形態がいちじるしく異なる場合があるので、これらをホンシメジA、B、CおよびDの4型に区別した。このうちA、CおよびDはそれぞれ *Tricholoma aggregatum*, *Agaricus amplus* および *A. decastes* として、すでに与えられた学名が示すものと同一物のようである。



第1図版 1) ホンシメジA, $\times 1/2$. 2) ホンシメジB, a および b : 菌糸塊, $\times 1/2$. 3) ホンシメジBの菌糸塊, a : その断面, $\times 1/2$. 4) ホンシメジC, $\times 1/2$. 5) ホンシメジCの菌傘表面に生じた多数の菌蕾, $\times 4$. 6) ホンシメジD, $\times 1/2$. 7) シャカシメジ, $\times 1/2$.

- 2) これら4型はいずれも4極性である。 全ての組み合わせにおいて癒合がみられない。したがって、両者は分類学上別種とみなすのが妥当である。
- 3) AをB, CおよびDのそれぞれと交配した結果は、すべての組み合わせにおいて癒合がおこる。したがって、これら4型は同一種とみなすのが妥当である。
- 4) シャカシメジも4極性である。そして地方的な形態変化はほとんどみられない。 種々ご助言、ご教示をいただいた京大助教授浜田稔博士に深く感謝する。なお、貴重な文献の調査に關し山口大教授御江久夫博士から多大のご援助をいただいた。ここに衷心拝謝する。
- 5) ホンシメジAとシャカシメジの間では、す

文 献

- 1) 今関六也, 林試研究報告 57: 141 (1952). 2) 広本一由, 植雑 73: 326 (1960). 3) 西門義一・木村勘二, 農学研究 25: 474 (1935). 4) 河村栄吉, 九大農芸雑誌 9: 337 (1941). 5) 伊藤一雄, 林学会報 26: 185 (1944).

Summary

1. The results of the ecological and morphological studies on *Tricholoma aggregatum* collected from nine stations reveal that the samples are classifiable into four morphological types; they are, for convenience' sake, called type A, B, C and D.

2. From the results obtained from all the possible pairings of monosporous mycelia isolated from these four types (Tables 2, 3, 4 and 5), it may be concluded that these four types are all tetrapolor. And it is more reasonable to regard that these four types are of the same species, because the results of the experimental crossing reveal that the monosporous mycelia of type A can conjugate with those of each of other three types as shown in Tables 7, 8 and 9.

3. *T. conglobatum* is tetrapolor, too (Table 6). In it any local and morphological variation can be hardly observed.

4. *T. aggregatum* and *T. conglobatum* should be classified into different species, because no conjugation of the type A of *T. aggregatum* with *T. conglobatum* can be observed in experimental crossing (Table 10).

Short Communication

Yutaka MURAKAMI*: Formation of Gibberellin A₃ Glucoside in Plant Tissues

村 上 浩*: 植物組織におけるジベレリン A₃ グルコシドの生成

Received June 26, 1961

Although the physiological action of gibberellin A₃ has been extensively studied in recent years, no work has been done about the fate of this compound in the tissue of higher plants. The author observed that exogenous gibberellin A₃ is converted in many plants into a glycoside which gives a blue color with Folin-Ciocalteu reagent and induces a growth response in the rice seedling test. This paper describes the experiment with young cucumber leaf disks.

Young cucumber leaf disks, 1.5 cm. in diam., were floated on a solution containing 1,000 ppm gibberellin A₃ in 1% sucrose and placed in the light at room temperature. After 3 days, 200 g. of the disks were removed from the solution, washed with water, and extracted two times with 1 l. of 70% acetone. The filtered extract was concentrated under reduced pressure. The aqueous residue was then adjusted to pH 2.5 with phosphoric acid and shaken five times with twice its volume of ethyl acetate in order to remove free gibberellin A₃. The resulting aqueous fraction was extracted three times with 100 ml. of *n*-butanol. This butanol extract was dried over anhydrous sodium sulfate, concentrated *in vacuo*, and the residue was spotted on chromatography paper. Chromatograms were run in the solvent system of *iso*-propanol/ammonia/water (10:1:1). A chromatogram sprayed with Folin-Ciocalteu reagent showed a blue spot characteristic of gibberellin A₃ at R_f 0.4 where gibberellin activity was detected. Under the same conditions of development, free gibberellin A₃ was located at R_f 0.6. For the identification of this new compound, the zone corresponding to R_f 0.4 was eluted with water, the eluate was concentrated, and chromatographed again with *n*-butanol/glacial acetic acid/water (4:1:2). One half of the chromatogram run from the spots was assayed by the rice seedling test and the other half was sprayed with Folin-Ciocalteu reagent. After spraying, a definite blue spot appeared at R_f 0.68, corresponding to the growth-promoting zone on the chromatogram (Fig. 1A, D). The R_f value of gibberellin A₃ was 0.9 in this solvent system. Then from a number of chromatograms this new gibberellin was eluted with water, and hydrolyzed with either 1 N hydrochloric acid at 100° for 1 hour or emulsin (β -glucosidase) at 30° for 1 day. The hydrolysis products were concentrated and chromatographed in *n*-butanol/acetic acid/water, respectively. On the chromatogram of the acid-hydrolysis products glucose was detected with benzidine-trichloroacetic acid reagent. After treatment with emulsin, paper chromatography revealed the presence of glucose and gibberellin A₃ (Fig. 1B, C). This fact indicates that the compound formed is a β -glucoside of gibberellin A₃. Similar gibberellin A₃ derivatives were also obtained with young leaves of the following plant species: *Pharbitis Nil* (Japanese morning-glory), *Ipomoea Batatas* (sweet potato), *Diospyros Kaki* (Japanese persim-

* National Institute of Agricultural Sciences, Nishigahara, Kita-ku, Tokyo, Japan.
農林省農業技術研究所

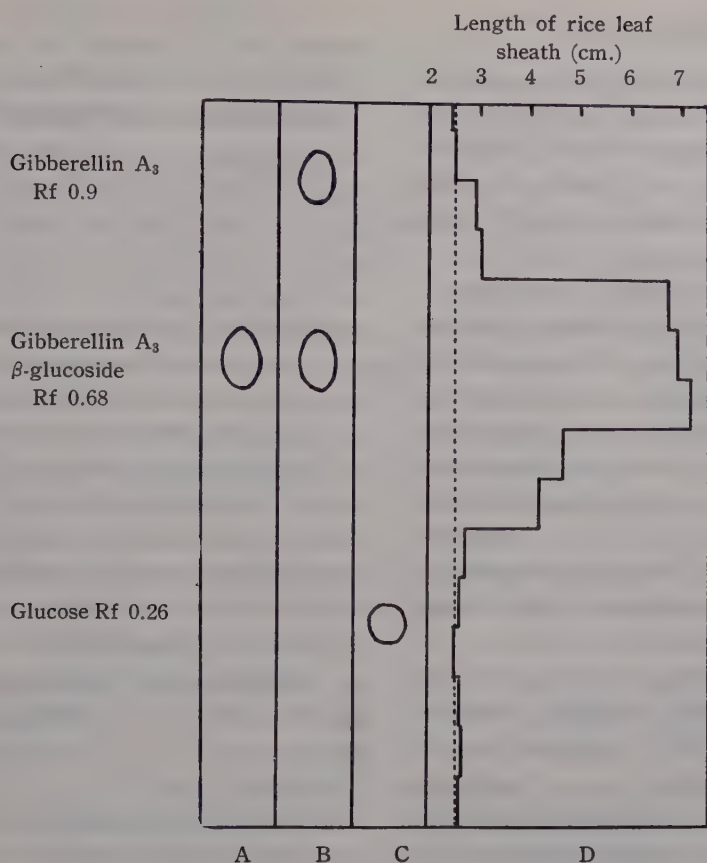


Fig. 1. Paper chromatograms showing the formation of gibberellin A_3 β -glucoside by treating cucumber leaves with gibberellin A_3 .

Ascending paper chromatography using Toyo No. 50 filter paper. Solvent; *n*-butanol/glacial acetic acid/water (4:1:2), distance travelled; 29 cm, temperature; 22~25°. A and B were sprayed with Folin-Ciocalteu reagent¹⁾, C with benzidine-trichloroacetic acid reagent, and D bio-assayed by rice seedling test²⁾.

mon), *Arachis hypogaea* (peanut), *Lupinus luteus* (yellow lupine), and *Pyrus* Simonii (pear).

The formation of glycosides in plant by adding different aglucones is a well-known phenomenon and is considered to be a detoxication mechanism. A similar role might be played by the conjugation of gibberellin A_3 with glucose in plant tissues.

References

- 1) Udagawa, K., and Kinoshita, S., Jour. Agr. Chem. Soc. Japan 35: 224 (1961).
- 2) Murakami, Y., Bot. Mag. Tokyo 70: 376 (1957).

書 評

Hawkes, A. D.: Orchids, Their botany and culture, pp. 297. Harper and Brothers, New York (1961). 著者はフロリダに住み、蘭をたずねて世界各地を旅行し、現在 *The Orchid Journal* および *The Orchid Weekly* の主筆で、この種の書物にはうってつけである。

洋蘭をはじめの人への手引として格好な書物、らん科の概説、栽培の要点をのべた後で主な温室向きの種類を、属のABC順にならべ、相当数の写真や描画を入れて簡潔にのべている。これはいわゆる原理であって、そのほかに、品種や交配雑種のリストもある。巻末に *Schlechter* の分類系で排列した 90 亜族 668 属の分類表をつけたのは蘭の愛好家以外にも便利であろう。(前川文夫)

Zimmermann, K. F.: Praktische Pflanzenzüchtung, pp. 231. VEB Gustav Fischer Verlag, Jena (1961) (定価 18.30 マルク)。

品種改良は新種を育成し、それを増殖することである。農業生産は作物を労働の対象として農産物をつくりだすのであるから、育種は農業生産において原料を供給する立場にあるといえる。農業生産が国家経済において果たす役割りは大きいから、作物の改良の目標は、そのときの国家経済の動きによっていちじるしく左右される。本書は社会主義国家であるドイツ民主共和国(東独)における育種学の教科書として書かれたものであるが、計画経済のもとの育種のあり方について深い考察がなされている。

著者のチンメルマン教授はベルリンのフンボルト大学において、植物育種学、遺伝学および生物統計学の講座をうけもち、古くから育種技術の改善を研究分野としてきた人であり、その豊富な知識と経験から植物育種学をかきあげている。

本書は全体が9章にわけられ、それぞれつぎのような内容の順に配列されている(カッコ内はページ数)。

1. 序論(4)
2. 植物育種の歴史(11)
3. 植物育種の目的(13)
4. 植物育種の理論的基礎(26)
5. 植物育種の方式(79)
6. 植物育種の技術(68)
7. 植物育種の組織(21)
8. 植物育種の効果(5)
9. 植物育種の将来(5)

ページ数でわかるように、著者がもっとも力を入

れているのは5章から7章にかけてであり、本書が技術者をも対象としてかかれていることをしめしている。以下章を追ってその内容の輪郭を紹介する。

1章から3章にかけては、植物育種の定義、歴史および目的を論じている。植物育種は植物にその性質をたかめる能力をあたえる行為であり、収量が多く品質のよい品種や系統を農民に供給し、国民の生活水準をよくするための手段であるとのべている。

4章では、植物育種の方式をくみだてるのに必要な基礎的原理について一通りの説明をおこなっている。このなかで、リュセンコ説についても言及しているが、現在のところこれを否定する成績がおおいこと、また遺伝学は形式主義的な固定的な科学ではなく、たえず進歩をもとめている学問であることをのべて、リュセンコ説を批判している。

5章では、植物育種の方式についてのべている。8種類の育種法をとりあげ、模式図によってわかりやすく説明している。

本書の特色は6章の植物育種の技術にあるとおもわれ、育種技術者の教育、育種事業に必要な装置や機械についてくわしく論じ、農業の機械化とともに育種事業の機械化ももっととりあげられるべきであることを指摘している。

7章では、東独における植物育種の組織についてくわしい紹介がなされている。

8章では、世界的な人口増加に対処して育種のあり方を論じ、過去において育種が農業生産にどれだけ貢献したかを考察している。

9章では、植物育種が人為的におこなう進化であるという考え方から、将来の研究課題として人為的に遺伝的变化をおこす、X線による突然変異やコルヒチンによる倍数体の誘発をあげている。さらに、今後の育種事業は共同研究や合理的な労力配分によって能率よくはこばねばならないが、これは育種事業の国有化により可能であろうと結んでいる。

本書でも、育種において対象とする形質が、質的形質ではなく量的形質であることを強調しているが、量的形質の選抜においてもちいられている統計法についても言及すべきではなからうか。いずれにせよ、本書は植物育種をあらたな観点からとりあげたものとして注目され、植物育種に関心をもつ人たちへのよい手引きになるとおもわれる。

(山口寿之)

抄 録

カサノリの種特異的なタンパク質の合成
における核と細胞質との間の相互作用

Keck, K.: Nucleo-cytoplasmic interactions in the synthesis of species-specific proteins in *Acetabularia*. Biochem. Current Literatures and Biophys. Res. Communications 3: 56—61 (1960).

最近、カサノリでは除核部でもかなりの量のタンパク合成がおこなわれうることが知られた。また種々の酵素、すなわち、高度に特異的なタンパクが核がなくても生成するという証拠もある。したがって核によるタンパク合成の制御はむしろ間接的なもので、細胞質は核に由来した遺伝情報を、それ自体でもかなり高度に保持できるもののように思われる。

ここでは種特異的な酵素タンパクの構造を決定するのに、核および細胞質のどちらの要因が大きいかを追求された。Starch gel electrophoresis によって *Acicularia Schenckii* と *Acetabularia mediterranea* の2種から得られた acid phosphatase が、それぞれ泳動性に顕著な型を示し、*med*-型が *acic*-型より strip 上の移動が速く、両者の混合試料でもじゅうぶんに分離できることが確かめられた。この酵素は核を含む仮根を切断すると、茎ではその生成が急速に低減するが、核なしでもしばらくは構造的にも機能的にもまったく正常な酵素タンパクがつくられる。無核の *acic* の茎を、核を含む *med* の仮根につぎ木する (*acic₀-med₁*) と、はじめは *acic*-型 phosphatase がつくられるが、2日目からは *med*-型のバンドが現われ、5日後には両型が完全に入れ変わって *acic*-細胞質の酵素生成は *med*-核の制御の下におかれてしまう。しかし逆つぎ木 (*acic₁-med₀*) の場合にはその逆にはならない。そこで両核を含んだつぎ木 (*acic₁-med₁*) をつくと、例外なくすべて *med*-型のバンドが starch strip 上に現われ、*med*-型が *acic*-型より “dominant” であることがあきらかになる。しかし核なしでも *acic*-茎は35日間も *acic*-特異型酵素を、わずかではあるが合成しうるのである。結局、タンパク質構造をつくるには核、細胞質の両要因がともに責任を負うことをしめしており、これらの結果は文頭に記した推測の裏づけとなるものである。(吉田吉男)

シダの蔵精器分化を誘導する物質

Näf, U.: (A) On the physiology of antheridium formation in ferns. Proc. Fourth Intern. Conf. Plant Growth Regulation, 1959: 709—723 (1961). (B) Mode of an antheridium-inducing substance in fern. Nature 189: 900—903 (1961).

さきに Döpp (1950) はシダの前葉体から抽出したある種の物質が、同種および異種のシダ前葉体の蔵精器分化を誘導することをみた。上記の二論文はこの問題に関するよりくわしい研究の報告である。

(A) 前葉体の発生には二つの段階があり、前段階で蔵精器が分化し、後段階では蔵精器分化が止まって、かわりに蔵卵器が分化する。さきの誘導物質は両段階を通じて前葉体内に生産されるが、その濃度は後段階で最高に達する。*Pteridium* では、蔵精器の分化に対しては前葉体からとり出した誘導物質の標準濃度の $1/2 \sim 1/50$ の範囲がもっとも有効であるが、これは前段階で自己が生産するこの物質の濃度以上である。したがって、蔵精器の分化は自己の前葉体が生産した物質によって誘導されるのではなく近くにある、より成熟した前葉体から分泌する誘導物質によると考えられる。その証拠には、単独に培養すると蔵精器分化はおこらない。(B) *Onoclea sensibilis* の前葉体は通常の培地では蔵精器をつくらないが、*Pteridium* から抽出した物質をあたえると蔵精器を分化する。また若い前葉体から分裂組織を含まないように翼部を切りだしてそれを通常の培地におくと、それだけでも 55 % の割合で蔵精器を分化する。もし分裂組織が混在すると通常の培地ではこの分化がおこらない。切り出した翼を誘導物質を加えた培地におくと蔵精器分化の割合は 75 % に上昇する。正常の前葉体に一時的に原形質分離をおこさせてから通常の培地へもどすと、高い率で蔵精器を分化する。これらの事実から、通常に分裂組織の活動が蔵精器分化を抑制しているが、原形質分離によって細胞間の連絡が断たれるとその抑制が開放されて蔵精器が分化するとみられる。誘導物質の作用機構は分裂組織の活動を消却することによって、この抑制を開放するにあると考えられる。誘導物質の化学的正体は未知であるが、pH 2 で 10 分間煮沸しても破壊されないが、pH 12 で 10 分間煮沸すると破壊される。(中沢信午)

本 会 記 事

新 入 会 員

(昭和 36 年 7 月 31 日まで)

北海道支部

刘 逸民 北大理植 (札幌市北 22 条西 9 丁目18)

東北支部

渡辺 信 大曲市中道東22秋田県立大曲農業高校
(秋田県大曲市中町19)山本 弘 岩手県宮古市八幡前宮古高校 (岩手県
宮古市八幡前40)

岡上 伸雄 東北大理生物 (仙台市中江町南13の71)

関東支部

浜田 裕一 横浜市港北区日吉本町日吉台中学 (横
浜市磯子区森町1460)

西山 岩男 東大理植 (杉並区阿佐ヶ谷 3 の254)

橋本 保 東大理付属植物園 (板橋区栄町 7 細
井方)長沢 幸雄 山梨県南巨摩郡増穂町増穂中学 (山梨
県中巨摩郡櫛形町桃園77)遠山 順一 掛川市神明町県立掛川東高校 (静岡県
掛川市秋葉通り)岡村 実 世田ヶ谷区下馬 3 の35 東京学芸大付
属高校 (武蔵野市境744)井上 晃男 東大農水産植物 (世田ヶ谷区北沢 3 の
895)

許 建昌 東大理植

和田 優 豊島区池袋 大東文化学園大学 (八王
子市瀬田町237)

美濃 羊輔 東大理植 (杉並区阿佐ヶ谷 3 の254)

尼子富士郎 杉並区上高井戸3—848 医学中央雑誌
社 (杉並区上高井戸3—848)

永井 静江 都立大理生物 (目黒区衾町42 栗原方)

服部 明彦 東大応微研10研 (世田ヶ谷区玉川等々
力 3 の60)武田 幸作 東京教育大理植 (杉並区馬橋 2 の 164
中鉢方)池田 泰治 東京教育大理植 (豊島区池袋 6 の1965
酒井敬之方)

中部 支部

持塚 洵 静岡市水落町 1 の 1 ポーラ化成工業
研究所大滝 保 名大理生物 (名古屋市瑞穂区中山町 2
の19 原もと方)

北陸支部

城代 辰生 金沢大理生物

原田 俊光 金沢大理生物

本多 啓七 富山県魚津市吉島県立魚津高校 (富山
県黒部市三日市3361)

近畿支部

平田 保 滋賀県甲賀郡甲賀町五反田塩野義製薬
油田農場 (京都市左京区下鴨東森ヶ前
町 2)

中国・四国支部

高山 敬三 岡山県勝田郡奈義町奈義中学 (岡山県
勝田郡勝北町中村274)御舟 政明 鳥取県三朝町岡山大温泉研 (鳥取県三
朝町三朝)

野川 茂 (鳥取県西伯郡淀江町973)

山木 輝男 島根大文理生物 (島根県八束郡宍道町
西来待309)

九州支部

西林 茂人 鹿児島市高麗町27鹿児島実践女子高校
(鹿児島市高麗町18 石埼方)小池 勝 佐賀県鹿児島市高津原鹿児島実業高校 (佐
賀県佐賀市上多布施町昭栄アパート)沢井 勉 長崎県平戸市紐差町県立猶興館高校紐
差教室 (長崎県平戸市津吉町1139 岩
元岩吉方)

外 国

権 五溶 韓国京城特別市京城大学文理科大植

朴 萬奎 大韓民国京城特別市科学博物館

鄭 濬 韓国大邱市慶北大学校文理科大

楊 麟錫 韓国大邱市慶北大学校文理科大

住 所 変 更

(昭和36年7月31日まで)

北海道支部

松川 浩彬 虻田郡俱知安町俱知安中学

東北支部

岩田 悦行 (盛岡市上田緑ヶ丘住宅12号)

大森 和彦 宮城県栗原郡築館町築館女子高校 (宮城県栗原郡築館町仲町15 佐藤方)

高松 正彦 弘前市下白銀町1 弘前大教育生物

関東支部

小野 貞雄 長野県北佐久郡浅間町 長野県立岩村田高校

福本 日陽 府中市東京農工大一般教育部

山田 保 (千葉県木更津市牛袋183)

金子 善彦 神奈川県茅ヶ崎市県立茅ヶ崎高校 (神奈川県茅ヶ崎市菱沼1693 古知屋忠五郎方)

橋本 徹 武蔵大学(北多摩郡保谷町上保谷1845)

吉田 幸弘 立川市曙町3—216 都立立川短大生物 (埼玉県入間郡福岡町霞ヶ丘117—1)

宮口 証子 山口証子(改姓)(足立区西新井468 西新井団地11号404)

柴岡 弘郎 東大理付属植物園(北区王子町1160 王子アパート509号)

松崎 悦三 千葉県習志野市大久保 東邦大理生物

星川 清親 北多摩郡田無町東大付属農場

島村 環 横浜市大文理生物 (鎌倉市雪の下915)

野村 清 新宿区戸塚町3—1000 新宿区立西戸山中学 (豊島区池袋1—122 高橋順市方)

佐藤 茂 新潟市川岸町2 白新中学

向坂 道治 (田無局区内保谷町上保谷688)

浅井 康宏 (世田ヶ谷区玉川奥沢町3の185)

坂崎 信之 (千葉県千葉郡八千代町大和田新田760)

柴田 寛三 世田ヶ谷区世田ヶ谷4丁目 東京農大

中部支部

藤田 達也 (静岡県掛川市瓦町)

香田 寿男 岐阜県揖斐郡久瀬村立公正小学校

吉井 義次 (名古屋市千種区徳川山町3の7)

須賀 瑛文 名古屋市東区筒井町名古屋市立あずま中学 (名古屋市千種区代万町3の11 虹丘中団地14号館204号)

北陸支部

柴田 万年 富山大文理生物

近畿支部

桑名 誉 関西学院大理生物 (吹田市西之庄町517)

田中長三郎 (神戸市東灘区住吉町瀬川1324)

亀谷 嘉夫 (岐阜県可児郡可児町東帷子)

桑田 義備 (京都市左京区銀閣寺町19)

田中美智子 村尾美智子(改姓)(大津市膳所池の内887)

椿 啓介 大阪市東淀川区十三西之町財団法人酸酵研 (大阪市東淀川区加島町371 武田社宅23号)

並河 功 (大阪府寝屋川市太秦37の7)

森 祐二 奈良県橿原市奈良医大細菌

森本 哲郎 京都府亀岡市横町府立亀岡高校

遠藤 信雄 (京都市北区柴野東船岡町18 岡田義治方)

吉川 知之 大阪府池田市畑町 池田高校

俵 勝也 大阪市福島区鷺州上通2の47

中国・四国支部

渡辺 義生 (広島県安芸郡船越町引地1944)

山本 正明 広島県安芸郡府中中学

沢良木庄一 (高知県中村市入田3205)

三原 勉 松山市梅津寺町1177 松山市立高浜中学

横村 英一 山口県宇部市小串山口県立医科大第一生理

広江三樹三郎 (倉敷市天城601)

西上 一義 松江市西川津町 島根大文理生物

浦山 隆司 (岡山県倉敷市老松町388 藤原時夫方)

守田 治夫 山口県岩国市錦見県立岩国商業高校

岡 嘉明 島根県益田市大字白山市立中西中学

九州支部

河野 明綱 (宮崎市権現町161)

細川 隆英 (福岡市田島池の田1433の4)

坂口 信治 鹿児島県川内市川内高校

奥 達夫 九大理生物

大木 麒一 (熊本市神水町 391 の 4 前田潔志方)

小島 均 福岡市高宮玉川町第一薬科大

西原 幸男 福岡県築紫郡太宰府町太宰府高校

野沢ユリ子 鹿児島大水産

鴨川 誠 長崎県上県郡上県町佐護佐護中学

岡畑 寛 (熊本県玉名市繁根木184 長谷川茂作方)

外国

島袋 守成 沖縄島美里村ゴザ高校

韓 昶烈 韓国全北大学校農科大遺伝育種

ラオス王国自然科学協会創設について

このたび、ラオス王国自然科学協会 (Société Royale des Sciences Naturelles du Laos) がビエンチャンに創設されたむね、同協会長より外務省と文部省を通じて通知がありました。同会はラオスの動植物および鉱物の研究に興味をもつものを全面的に歓迎し、物的、財的援助の供与を期待しています。

会 員 名 簿 (昭和 36 年 1 月号登載) 訂正

ページ

誤

正

5 浦口直佐 港区芝功運町 30 普連土学園 浦口真左 港区芝三田功運町 30 普連土学園
(渋谷区伊達町 21 柴沼方)

18

賀来輔章

賀来章輔

訂

正

7-8月号木村氏論文に係の下手際から誤りがありました。おわびをして訂正いたします。

364 ページ Table 4 の $35^{\circ} \rightarrow 25^{\circ}$.

同, 上から 7, 9, 10 行目の coty-ledon(s) \rightarrow cotyledon(s).

文献 4) の Bot. \rightarrow Z. Bot.

同 12) の Cajlachan \rightarrow Cajlachjan.

Developmental Mechanics of Fucaceous Algae XIX. Negation to the Presence of Polar Cyto-skeletal Systems in the Endoplasm of *Coccophora* Eggs*

by Singo NAKAZAWA**

Received June 20, 1961

In *Fucus furcatus*¹⁾ and *Cystoseira barbata*²⁾, the developmental polarity of the egg is determined by means of centrifuging. But in some other fucoids, such as *Coccophora Langsdorfii*³⁾, *Sargassum confusum*³⁾, *S. tortile*³⁾ and *Fucus serratus*⁴⁾, it is not determined by centrifugal force. Invalidity of centrifugation to the polarity determination was also reported in *Equisetum* spores^{5,6)}, *Vaucheria* spores⁷⁾, and in animal eggs. For explaining the stability of the developmental polarity as in the latter cases, Lillie⁸⁾, Conklin⁹⁾ and others presumed the presence of an immovable polar skeletal system pervading the endoplasm of the egg. This hypothesis has been attacked by discovery of the presence of cortical cytoplasm that is also stable even for a strong centrifuging. Further, the fact of protoplasmic stream will be another evidence that opposes the presence of endoplasmic skeleton. If there were a polar skeletal system in the endoplasm, it would obstruct differentially in directions the movement of intracellular elements when the cell is centrifuged. As a result, the appearance of stratification would be more retarded towards a certain direction than towards another according to the orientation in which an egg undergoes centrifugation. In addition, once the intracellular elements were stratified, velocities of their redistribution would also differ with directions. In this idea, Howard¹⁰⁾ made experiments with sea urchin eggs, and obtained negative results. The present writer undertook a series of similar experiments on fucoid eggs.

Material and Methods

The material was *Coccophora Langsdorfii* collected in Asamushi in April, 1961. Eggs were fertilized artificially in glass vessels by usual method. The egg is spherical or a little elongated before and just after fertilization, and later it is transformed by morphogenetic movements into an ovate form pointed towards one end. Morphologically, the developmental polarity is determined at the later stage. At the two different stages, eggs were centrifuged with various centrifugal forces. An electric centrifuge, 10 cm. in radius, was used for obtaining 400 to 1,100 times gravity, and an air-turbine centrifuge of 1.5 cm. in radius for 25,000 times gravity. The time of centrifugation was 60 seconds for each. Observations with a microscope were divided into three points. (a) Whether or not there is a difference in velocity of stratification with the same centrifugal force between the stages before fertilization and after the determination of morphological polarity. The velocity of stratification was indicated in percentage of appearance of clear strata. (b) Whether or not there is a difference in velocity of stratification according to the direction in which the egg is centrifuged with the same centrifugal force at the same stage. The direction of

* Supported by a grant from the Saito Gratitude Foundation, Sendai.

** Biology Department, Yamagata University, Yamagata, Japan.

centrifugal force towards which an egg undergoes centrifugation is determined by chance when the egg is placed in the centrifuge. So that, the stratification can take place in various directions with respect to the polarity axis of the egg by chance. The velocity of stratification was measured in percentage of individuals exhibiting very clear strata. The percentage was calculated in each of the apical, the basal, and the lateral stratifications separately, excluding stratifications in intermediate directions. (c) Whether or not there is a difference in velocity of redistribution of the stratified elements according to the direction of centrifugation at the stage after the polarity was determined. The velocity of redistribution was measured in percentage of individuals retaining the clear strata. That is, the lower is the percentage, the higher is the velocity of redistribution. The percentage was calculated also in each of the apical, the lateral, and the basal stratifications, separately. The stratified layers should be observed right sideways to avoid indistinctness of their border-lines. Ratio of the replacement of nucleus to the central part of the egg cell was also measured for the same purpose. In this case, the higher is the ratio, the higher is the replacement velocity.

Table 1. Stratification ratio in *Coccophora* eggs when centrifuged at various forces for one minute.

Centrifugal force $\times g$	% of individuals exhibiting clear stratification patterns	
	Unfertilized eggs	Polarity-determined eggs
400	0	0
1,000	0	0
1,700	0	0
2,200	8	8
2,700	50	51
4,000	100	100
11,000	100	100

Results with Discussion

(a) As shown in Table 1 and also in Fig. 1, the endoplasmic elements cannot be stratified at a force lower than $1,700 \times g$ (=times gravity) regardless of the developmental stage. At $2,200 \times g$ rate of the clear stratification was 8 per cent. At $2,700 \times g$ it rose to 50 per cent, and at a force higher than $4,000 \times g$ almost 100 per cent eggs were completely stratified. These indicate that the limit of centrifugal force to stratify the cellular inclusions is between $2,200$ to $2,700 \times g$. It is noteworthy that there is no difference in velocity of stratification between stages so far as tested. If it were that a peculiar skeletal system were developed in the endoplasm after fertilization and it caused the polarity, the limit of centrifugal force necessary for the stratification should be different between stages before and after the appearance of developmental polarity, because the skeletal structure would obstruct movements of inclusions. The difference, however, could not be perceived. This implies that a cyto-skeleton can be rejected from the endoplasm. (b) If the egg is centrifuged at a stage after the polarity was determined morphologically, the limit of centrifugal forces for the apical, the basal, and the lateral stratifications are almost equal. That is, the ratio of individuals exhibiting clear stratification is 8 per

Table 2. Retaining of clear stratification six hours after centrifugation in eggs with determined polarity.

	Stratification		
	Apical	Basal	Lateral
Retaining %	21	20	23
Individuals inspected	86	93	92

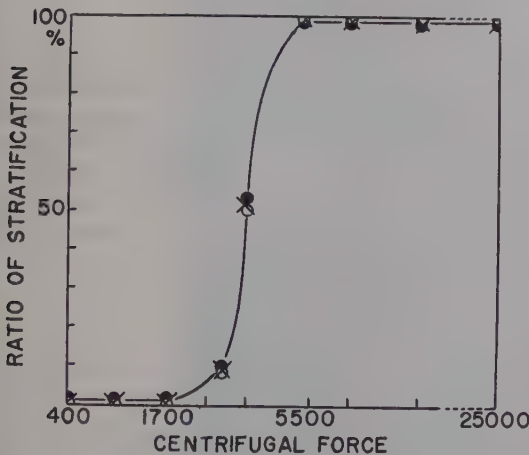


Fig. 1. Stratification ratios towards apical (—○—), basal (—●—), and lateral (—×—) directions when the egg is centrifuged after their polarity axis was determined morphologically. The ratio is represented in percentage of individuals exhibiting clear stratification patterns.

cent at $22,000\times g$, 50 to 53 per cent at $2,700\times g$, and almost 100 per cent at higher than $4,000\times g$, regardless of the direction in which an egg undergoes centrifugation (Fig. 1). These also indicate that development of the cyto-skeletal system cannot be considered even after the polarity was determined. (c) Modes of the redistribution of the stratified elements are different with stages. If an egg is centrifuged before fertilization and is left without being fertilized, all the visible elements stratified are retained without being redistributed. But, if it is centrifuged after fertilization, first the nucleus gets out of the plastid layer and is replaced to the central part of the egg cell within a few hours, then some of the plastids follow the trace of the nucleus and then are dispersed all over the endoplasm (Fig. 2). The oil cap and the free water layer are also diffused very soon. Most of the plastids are retained in the same layer until the egg gives rise to a young embryo, so that the centrifuged direction is distinguished with ease even at a later stage. Both the replacement of nucleus and the redistribution of plastids and other elements take place almost at the same velocity in any direction, basally, apically, or laterally (Fig. 2, Tables 2, 3). These also indicate that there is not a polar skeleton in the endoplasm. On this occasion, it will be questioned from the skeletal theory as to the possibility that the endoplasmic structure might be broken by strong centrifugation and the apolar redistribution might occur. This, however, is a contradiction. Because, if the cyto-skeleton is permitted, the stability of developmental polarity against a strong centrifugation should be based on the stability of cyto-skeleton, nevertheless the skeletal system is broken by the same centrifugation.

Hence, presence of a polar cyto-skeletal system should be denied regardless of the developmental stage before or after determination of the morphological polarity.

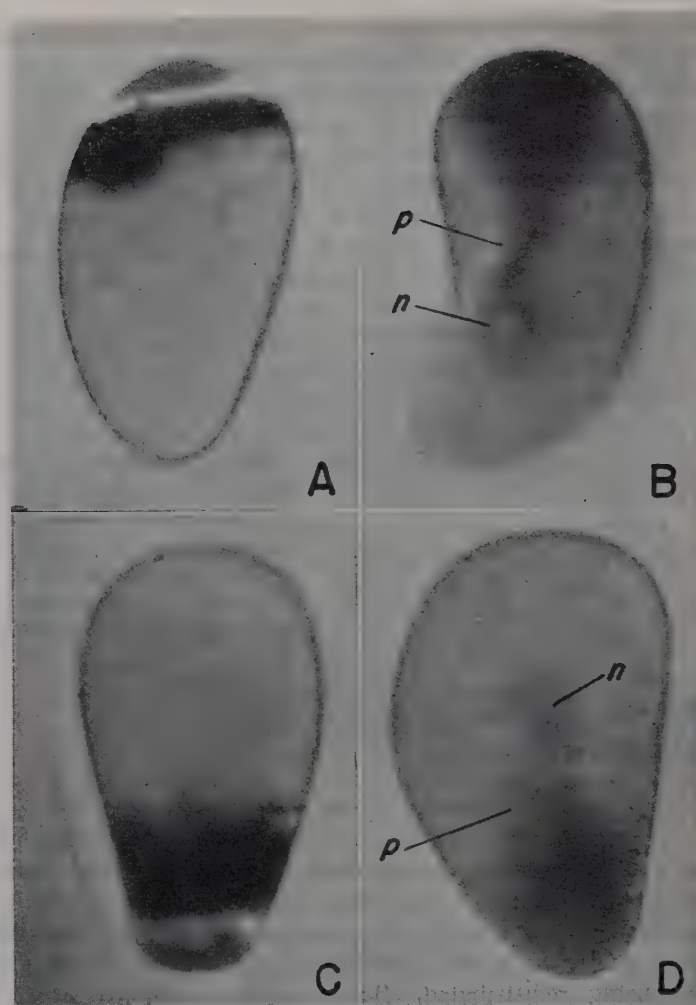


Fig. 2. Apical (A) and basal (C) stratifications in polarity-determined eggs, and their respective redistribution patterns (B, D) after six hours. n, nucleus; p, plastids.

Table 3. Replacement of nucleus six hours after clear stratification obtained with centrifugation at 4,000 times gravity, in eggs with determined polarity.

	Stratification		
	Apical	Basal	Lateral
Complete replacement %	79	80	77
Individuals inspected	86	93	92

Summary

Eggs of *Coccophora Langsdorfii* were centrifuged respectively before fertilization

and after determination of the morphological polarity towards various directions at 400 to 25,000 times gravity. As a result, the following was revealed. (1) The limit of centrifugal force necessary for stratification of the endoplasmic elements lay between 2,200 and 2,700 times gravity regardless of the developmental stage. (2) No difference was perceived in the velocity of stratification among directions, basal, apical and lateral, towards which the egg was centrifuged. The velocities of redistribution of the stratified elements also could not be distinguished with directions in which they were centrifuged. (3) From these facts, it is not considered that there is a polar cyto-skeletal system in the endoplasm of furoid eggs.

References

- 1) Whitaker, D. M., Biol. Bull. **73**: 249 (1937).
- 2) Knapp, E., Planta **14**: 731 (1931).
- 3) Nakazawa, S., Sci. Rep. Tohoku Univ. 4th Ser. **24**: 57 (1961).
- 4) Beams, H. W., J. Mar. Biol. Ass. Unit. Kingd. **21**: 571 (1937).
- 5) Nakazawa, S., Anal. Inst. Biol. Univ. Mexico **28**: 11 (1957).
- 6) Mosebach, G., Planta **33**: 340 (1943).
- 7) Weber, W., Z. Bot. **46**: 161 (1958).
- 8) Lillie, F. R., Biol. Bull. **16**: 54 (1909).
- 9) Conklin, E. G., J. Exp. Zool. **22**: 311 (1917).
- 10) Howard, E., J. Cell. Comp. Physiol. **1**: 355 (1932).

摘 要

中 沢 信 午: フークス科藻類の発生力学 XIX.
スギモク卵の中に極性の細胞骨格がないこと

スギモクの卵を、受精前および形態的に極性が決定してから、400~25,000 $\times g$ の間の種々の程度の遠心力で1分間遠心した結果、つぎのことが明らかになった。(1) 内部細胞質が層状化に必要な限界遠心力は、実験した発生段階に関係なく 2,200~2,700 $\times g$ の間である。(2) 卵の軸に対してどの方向に遠心力をかけても、同じ速さで層状化がおこる。また、一度層状化した内部細胞質要素は、卵軸に対してどちらの方向にもおなじ速さで分散していく。(3) これらの事実から、フークス科藻類の卵の内部細胞質に有極性の細胞骨格があるとは考えられない。(山形大学文理学部生物学教室)

Studies on the Mode of Action of 1:4-dihydronaphthoic acid-1 I.

Masayuki KATSUMI*

Received July 3, 1961

Many substances of chemically different natures are called auxins owing to their promoting effect on shoot elongation. It is doubtful, however, whether or not their primary actions in the cell are the same for all of them.

Some naphthoic derivatives have strong auxin activity,^{2,6,7,8} despite of their lack of the structural requirements proposed by Koepfli, Thimann and Went³). The purpose of the present study was originally to investigate whether 1:4-dihydronaphthoic acid-1 was a genuine auxin or not. Experimental results, however, suggested that the naphthoic acid derivative had something common with naphthaleneacetic acid in the primary action, and not with indole-3-acetic acid.

Material and Method

From 7-day old seedlings of Alaska pea, grown in a dark-room at 25°C, those having the third internode 15-20 mm. in length were selected. Discarding the apical 5 mm. of the third internode, the following 5 mm. zone was excised for experimental use. Ten of those sections were floated on about 15 ml. of test solution in a Petri dish and kept in the dark at 25°C. After 24 hours their lengths were measured under binocular microscope of low power with an objective micrometer of 0.1 mm. scale. The mean final length in the test solution was expressed as percentage of that in pure water. The results presented below are averages of at least two experiments.

Abbreviations 1:4-H₂-NcA, IAA, NAA, 3CIBA and 4CIBA stand for 1:4-dihydronaphthoic acid-1, indole-3-acetic acid, naphthaleneacetic acid-1, 3-chlorophenoxy-isobutyric acid and 4-chlorophenoxy-isobutyric acid, respectively.

Results

The effect of various concentrations of 1:4-H₂-NcA upon growth was found to be as shown in Fig. 1. The optimum concentration seems to be about 10 mg./l.

The results of mixing IAA in various concentrations with 10 mg./l. of 1:4-H₂-NcA are illustrated in Fig. 2. If the action mechanism were common to IAA and 1:4-H₂-NcA, the elongation in the presence of 1:4-H₂-NcA in the optimal concentration would not be increased by the addition of IAA in low concentration. Furthermore, the mixture in the optimal concentration of 1:4-H₂-NcA with IAA in the optimal and higher concentrations would depress the elongation as a supra-optimal effect, probably even to a level below that in the case with the single application of IAA. But neither of these expectations was found. Hence the two substances do not seem to act on the same site.

The elongation-concentration curve of NAA resembles that of 1:4-H₂-NcA, and the optimal concentrations of the two substances are roughly the same. When the two substances were mixed, the above-mentioned expectations based on the assump-

* Biology Department, International Christian University, Mitaka, Tokyo, Japan.

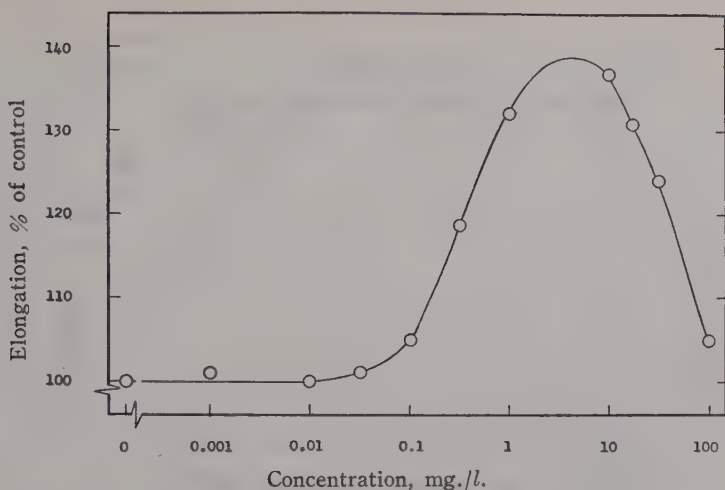


Fig. 1. Concentration effect of 1:4-dihydronaphthoic acid-1 on the elongation of pea stem sections. Final length in percentage of that of control.

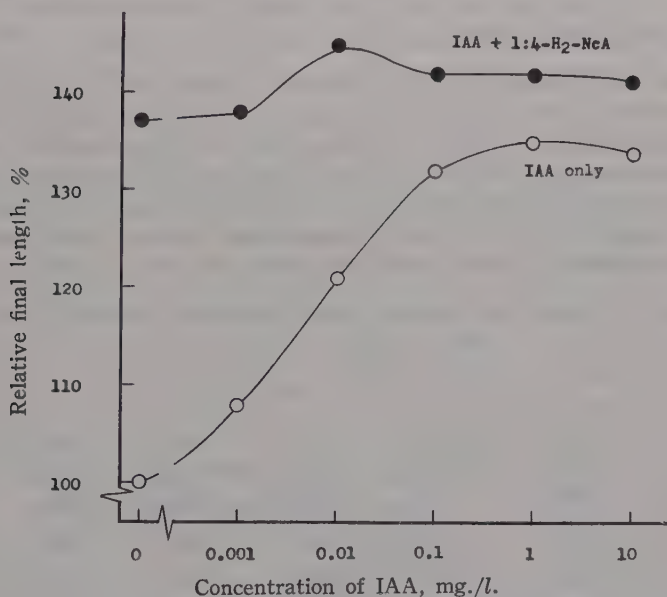


Fig. 2. Effect of indole-3-acetic acid, alone and in combination with 1:4-dihydronaphthoic acid-1 (10 mg./l.), on the elongation of pea stem sections. Final length of sections in percentage of that of control.

tion that they should act on a common reaction site were shown to be realized (Fig. 3). Namely, the effect of 10 mg./l. 1:4-H₂-NcA was not influenced by the addition of less than 1 mg./l. of NAA, but was suppressed by that of 10 mg./l. or more of NAA. Hence it is suggested that 1:4-H₂-NcA and NAA act in a common mechanism.

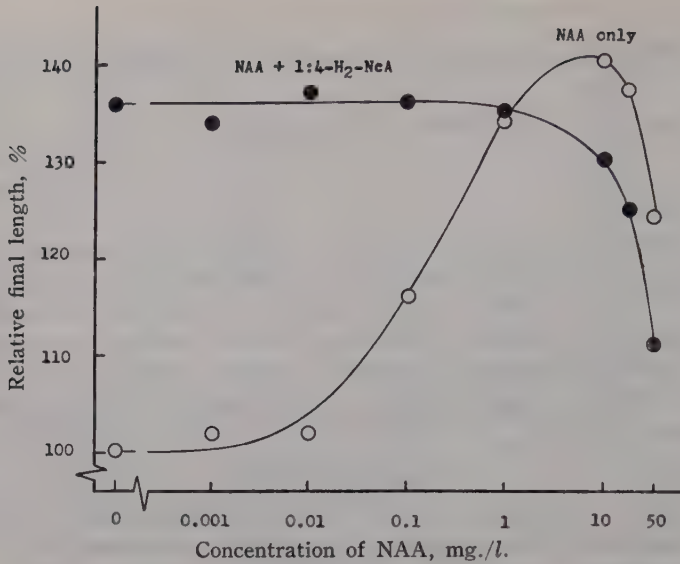


Fig. 3. Effect of α -naphthaleneacetic acid, alone and in combination with 1:4-dihydronaphthoic acid (10 mg./l.), on the elongation of pea stem sections. Final length of sections in percentage of that of control.

It may then be expected that NAA does not interact with IAA. In order to see the relation between IAA and NAA, they were mixed in various concentration ratios. The results are shown in Table 1. When one of the two auxins was in its sub-optimal concentration, the addition of the other one could increase the elongation. But the addition of IAA in the optimal concentration (10 mg./l.) did not intensify the supra-optimal effect of NAA. Thus it is suggested that the action mechanism both of 1:4-H₂-NcA and NAA is same, being different from that of IAA.

Table 1. Effect of IAA on NAA-induced elongation of pea stem sections. Final length in % of control.

IAA mg./l.	NAA, mg./l.					
	0	0.1	1	10	25	50
0	100	116	128	133	129	113
1	134	138	142	140	133	115
10	139	139	142	137	130	112

That 1:4-H₂-NcA is a kind of auxins different from IAA may further be substantiated if antiauxins effective on the one are not effective on the other. 3CIBA and 4CIBA have been reported to be antiauxins by MacRae and Bonner⁴⁾ and by Burström¹⁾. When each of them was added to IAA solution of various concentrations, the final concentration being 25 mg./l., they proved to be antagonistic to IAA, as shown in Figs. 4A and B. When, on the other hand, they were added to a concentration series of 1:4-H₂-NcA as shown in Table 2, they did not modify the elongation caused by 1:4-H₂-NcA.

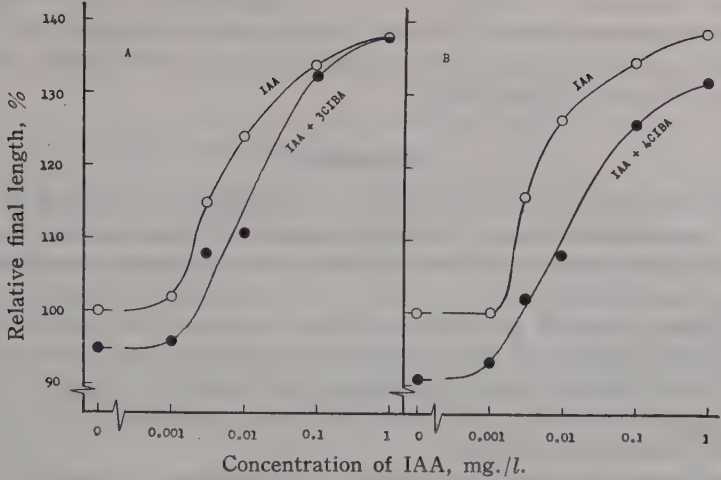


Fig. 4. Effect of 3-chlorophenoxy-isobutyric acid and 4-chlorophenoxy-isobutyric acid (25 mg./l. each) on the elongation of pea stem sections in the presence of various concentrations of IAA. Final length of sections in percentage of that of control.

Table 2. Effect of 3CIBA and 4CIBA on the elongation of pea stem sections in the presence of 1:4-H₂-NcA. Final length in % of control.

Compound mg./l.		1:4-H ₂ -NcA, mg./l.						
		0	0.001	0.01	0.1	0.5	1	10
3CIBA	0	100	—	100	105	124	130	136
	25	94	—	95	105	125	130	137
4CIBA	0	100	99	100	107	—	136	141
	25	96	96	97	107	—	136	141

Table 3. Effect of 4CIBA on NAA-induced elongation of pea stem sections. Final length in % of control.

4CIBA mg./l.	NAA, mg./l.				
	0	0.01	0.1	0.5	1
0	100	102	121	131	134
25	93	102	123	130	131

With a very high concentration, such as 50 mg./l., 4CIBA depressed the elongation effect of 1:4-H₂-NcA. But this does not seem to be attributable to an antagonistic effect of 4CIBA, since the same thing occurred also in combination with IAA.

As to the interaction of 4CIBA with NAA, experimental results were presented in Table 3. As expected, 25 mg./l. of 4CIBA did not show any appreciable antagonistic effect on NAA. Thus it was shown that the antiauxins used were antagonistic to IAA but not to 1:4-H₂-NcA and NAA.

Discussion

MacRae *et al.*⁶⁾ obtained straight lines in a double reciprocal plot of the growth against the concentration, using IAA, NAA and 2,4-D solutions or their mixtures. They discussed their results from the standpoint that the three auxin substances act on the same receptive sites.

In the present case of pea stem sections, however, the relation of growth to auxin concentration did not fit the equation for enzyme reaction. Hence the problem was studied by the additive effect of two auxins and by the interaction with antiauxin. The results suggest that NAA and 1:4-H₂-NcA act on a common site, which is somewhat different from the site for IAA.

It is not known by what mechanism the elongation is depressed by a supra-optimal concentration of auxin. Also it is not clear that the antiauxins used compete with IAA for the same site. Hence the experimental results reported above are not sufficient to discuss the action site of auxin substances. But it is to be noted that the two auxin substances having a naphthalene ring resemble each other and differ from IAA in the nature of their action.

Summary

1) Sub-optimal concentrations of indole-3-acetic acid (IAA) added to the optimal concentration of 1:4-dihydronaphthoic acid-1 (1:4-H₂-NcA) increased the elongation of etiolated pea stem sections, and the optimal concentration of IAA added to it did not cause the supra-optimal depression effect. The same was true when IAA was added to naphthaleneacetic acid (NAA).

2) Sub-optimal concentrations of NAA added to the optimal concentration of 1:4-H₂-NcA did not increase the elongation, and high concentrations of NAA added to it caused the depression effect.

3) 3-Chloro- and 4-chlorophenoxy-isobutyric acids were antagonistic to IAA but not to 1:4-H₂-NcA and NAA.

4) All results are consistent with the assumption that 1:4-H₂-NcA and NAA act on the same reaction site, but that IAA acts on a different site.

The author wishes to thank Professor Joji Ashida, Kyoto University, for his cordial guidance during the work and the preparation of this paper. Hearty thanks are also due to Mr. J. Kato, Kyoto University, for his helpful suggestion and support. The author is grateful to Dr. K. Koshimizu, Agricultural Department of Kyoto University, for the donation of 1:4-dihydronaphthoic acid-1.

References

- 1) Burström, H., *Physiol. Plant.* **7**: 241 (1954).
- 2) Kato, J., *Mem. Coll. Sci. Univ. Kyoto*, B **20**: 189 (1953).
- 3) Koepfli, J. B., Thimann, K. V., and Went, F. W., *J. Biol. Chem.* **122**: 763 (1938).
- 4) MacRae, D. H., and Bonner, J., *Physiol. Plant.* **6**: 485 (1953).
- 5) —, Foster, R. J., and Bonner, J., *Plant Physiol.* **28**: 343 (1953).
- 6) Mitsui, T., *J. Agr. Chem. Soc. Japan* **25**: 526 (1952).
- 7) Veldstra, H., *Rec. Trav. Chim. Pays-Bas* **7**: 15 (1952).
- 8) Zimmerman, P. W., and Hitchcock, A. E., *Contrib. Boyce Thompson Inst.* **12**: 321 (1942).

摘 要

勝見 允 行: 1:4-ジヒドロナフトエ酸-1の作用型に関する研究 I.

環とカルボキシル基との間に炭素原子をもたない諸物質のうちで、ナフトエ酸-1は弱いオーキシン作用を示す。さらにこの物質の水素添加誘導体は強い作用をもつ。これらのうちで作用性の顕著な1:4-ジヒドロナフトエ酸(1:4-H₂-NcA)をとり上げ、この物質の作用機構解明への一つの接近として、この物質とインドール酢酸(IAA)、ナフタリン酢酸(NAA)および抗オーキシンとの相互関係を調べた。

アラスカエンドウの黄化茎切片による伸長試験を利用した。

準最適濃度のIAAを最適濃度の1:4-H₂-NcAに加えると茎切片の伸長は促進された。さらに最適濃度のIAAを加えても超過濃度の影響はみられず、伸長促進作用は附加的となる。

この関係は、IAAとNAAについてもあてはまる。最適濃度の1:4-H₂-NcAに低濃度のNAAを加えた場合、伸長促進に変わりはないが、さらに高濃度のNAAを加えると伸長が阻害される。3-クロロおよび4-クロロフェノキシイソ酪酸はIAAに対しては抗オーキシンの働きを示すが、1:4-H₂-NcAおよびNAAに対しては作用を示さない。これらの諸結果は1:4-H₂-NcAとNAAとは同じ作用点に働くが、IAAは別の作用点に働くこと、つまり、前2者と後者とは作用機構がちがうことを暗示する。(国際基督教大学生物学教室)

Sexual Reproduction of *Chlamydomonas* as Affected by Ionic Balance in the Medium*

by Yoshihiro Tsubo**

Received July 18, 1961

Among environmental factors inducing sexuality in a green alga, *Chlamydomonas moewusii* var. *rotunda*¹⁾, depletion of assimilable N in the medium was found to be one of the important²⁾, and furthermore, light appeared to promote the mating activity³⁾. Although the mechanism of inducing sexuality is yet unclear, the observations agree with the general conclusion of many reports in the literature, beginning with Klebs⁴⁾, that the presence of light and the depletion of nutrient induce sexuality in a number of diverse algae⁵⁻⁸⁾. On the other hand, however, distilled water, in spite of its lack in N, does not make a medium more suitable for yielding abundant sexually functional cells than N-free synthetic medium²⁾. Other factors besides N in medium must therefore be involved in the induction of sexual activity. This paper concerns experiments performed to reveal the effect of some other factors, especially the ionic balance in the medium, on the sexual reproduction of this alga. An observation on the dedifferentiation of sexuality in a medium with assimilable N is also described.

Material and Methods

Organism: *Chlamydomonas moewusii* Gerloff var. *rotunda* Tsubo¹⁾ (used as *C. sp. 24* in previous experiments^{2,9)}) was originally isolated by the present author from Japanese soil. The organism is heterothallic and isogamous; two mating types are arbitrarily designated as + and -. This alga grows on an inorganic medium M*** under continuous illumination (ca. 2,000 lux) with fluorescent tubes (warm white) at 23°.

Mating of cells and estimation of sexual activity: When cells mate, they make *vis-à-vis* pairs; two cells are connected with each other by a protoplasmic bridge at their apical ends²⁾. The mating pairs swim for ca. 6 hrs., withdraw their flagella and fuse to form zygotes. Since sexually functional cells are morphologically analogous to non-sexual cells, the appearance of sexuality can only be recognized by the ability of producing mating pairs in the mixture of the two mating types. Therefore, for estimating the sexual activity, a drop of the cell-mixture was transferred onto a slide glass and fixed by a drop of acetocarmine, and then the frequencies of mating pairs and zygotes were counted under the microscope; a zygote was counted as equivalent to two gamete-cells.

In the present experiment for inducing sexuality, the following method²⁾ was ap-

* Contribution from the Biological Institute, Faculty of Science, Kobe University, Mikage, Kobe, Japan.

** Biological Institute, Faculty of Science, Kobe University, Mikage, Kobe, Japan.

*** Composition of medium M: NH_4NO_3 , 400 mg.; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 100 mg.; KH_2PO_4 , 100 mg.; $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 50 mg.; $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 10 mg.; $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$, 0.5 mg.; $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$, 0.39 mg.; H_3BO_3 , 0.6 mg.; $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 0.06 mg.; $\text{H}_3\text{PO}_4 \cdot 12\text{MoO}_3 \cdot 6\text{H}_2\text{O}$, 0.003 mg.; $\text{Na}_3\text{Citrate} \cdot 2\text{H}_2\text{O}$, 200 mg.; distilled water, 1,000 ml.

plied; vegetative cells of each mating type growing in medium M in the light were separately washed with distilled water, and equal amounts of each kind of cells were mixed together in a test solution of given salt concentration. The mixture was illuminated for 10~15 hrs., so that all the sexually functional cells produced in the mixture could accomplish mating. The mating frequencies shown in the following experiments represent the mean value of 2 to 4 parallel runs. All the cultures examined in this study were performed with a cell density of $10^5 \sim 10^6$ cells/ml.

Results

Dedifferentiation of sexuality in the presence of assimilable N: As reported in a previous experiment²⁾, when growing cells in a liquid medium were transferred to a N-free medium (M-N) and illuminated, sexual reproduction was induced, i.e. if the starved cells of each mating type were mixed together, cellular agglutination occurred, and a lot of mating pairs were eventually released; the growing number of mated gametes reached a maximum in about 2.5 hrs. after mixing (Fig. 1); the mating *per se* did not seem to require light.

In an experiment to demonstrate dedifferentiation of sexuality in the presence of assimilable N, illuminated gamete-cells of each mating type, obtained in M-N medium were separately inoculated into the following 3 kinds of media, i.e. M-N supplemented with NaCl, NH_4Cl , and NaNO_3 to a final concentration of 5 mM. each. All the cultures were placed in the light. At time intervals, equal amounts of the complementary mating types in each medium were mixed together and kept in the dark in order to avoid further development of mating activity by the light³⁾. After 2.5 hrs., mated pairs were counted under the microscope. As shown in Fig. 2, sexual activity of the cells incubated in the presence of assimilable N was evidently decreasing. Neither cell division nor appreciable enlargement of the cells was noticed during the 5 hrs. Nevertheless, when sexually functional cells of each mating type were plated onto M-agar, corresponding to cell counts in the original suspension, colonies were produced. This finding suggests that, if the gamete-cells were not allowed to mate

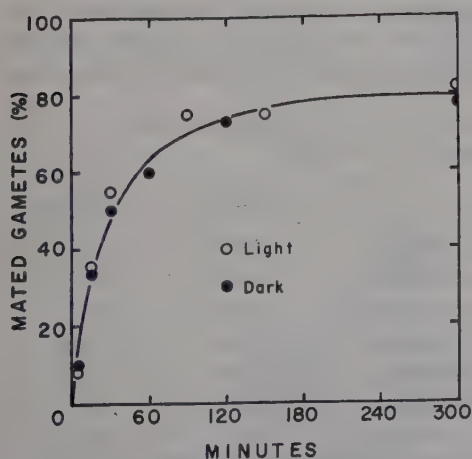


Fig. 1. Number of mated gametes formed on mixing two mating types of *C. moewusii* var. *rotunda* in the light or in the dark (conditions of experiment, see text).

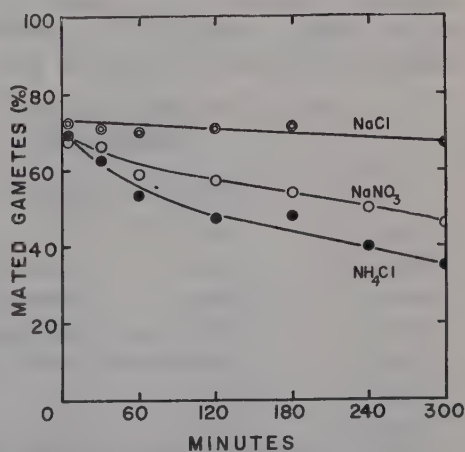


Fig. 2. Decrease in sexual activity in nitrogenous medium (salt concentration, 5 mM. each in M-N medium; conditions of experiment, see text).

and transferred to growing conditions, they could multiply as vegetative cells.

Thus, it is obvious that the assimilable N inhibits both gamete-formation²⁾ and/or mating of gametes. If the agglutination of gametes is supposed to be caused by the surface reaction of flagella^{9,10)}, the ionic constitution of medium might specifically influence this reaction, for instance, by interfering with the surface phenomenon in the presence of NH_4^+ or NO_3^- . On the other hand, the presence of N in the medium might have influenced the development of potential sexual activity in the cell on same nutritional ground. To elucidate the problem, the following experiments were planned to compare the effect of various ions on the induction of sexuality with that on the mating of gametes.

Induction of sexuality in single salt solution: Growing cells of both mating types were washed, mixed together and inoculated so as to make a population of 10^6 cells/ml. in each salt solution given in Table 1. Estimation of sexuality appeared in each solution was carried out after 15 hr.-incubation in the light. As shown in Table 1, sexuality in distilled water was less. Such cations as K^+ , Na^+ , and Mg^{++} promoted induction of sexuality. The depressing effect of NO_3^- on the activity of Na^+ and Ca^{++} is also clearly shown. Cells cultured in solutions of a higher concentration (50 mM.) of each salt were also examined, but all of them were more or less injured.

Saito¹¹⁾ described that ascus- or ascospore-formation of *Zygosaccharomyces major* was induced by the presence of such cations as Na^+ , K^+ , Mg^{++} , and Ca^{++} , added separately, or simultaneously as van't Hoff solution*. Therefore, the induction of sexuality in *Chlamydomonas* was examined with various concentrations of van't Hoff solution. The same procedures of incubation and testing were adopted for the use of van't Hoff solution. The increasing activity of sexual reproduction induced in van't Hoff solution up to 5 mM. might have been caused by the osmotic balance of the individual component ions. Therefore, the inductions of sexuality in various media lacking Na, K, Ca, Mg, S, or P were compared. For this experiment, 6 kinds of incubation media were prepared by mixing 2 ml. each of 4 stock salt solutions selected out of the following 7; MgSO_4 , MgCl_2 , KCl , CaCl_2 , K_2HPO_4 , Na_2HPO_4 and NaCl (5 mM. each). Fig. 4 shows that none of these 6 ions was indispensable for the induction of sexuality. The slightly less appearance of sexuality in the lack of K^+ or Ca^{++} might presumably have resulted from the ionic unbalance in the medium, especially since these two cations have strong antagonistic activities for controlling

Table 1. Frequencies (%) of mated gametes produced in inorganic salt solutions after 15 hr.-incubation in the light.

	0.5 mM.	5 mM.		0.5 mM.	5 mM.
LiCl	0	0	Na_2SO_4	12.6	45.5
KCl	19.8	42.1	MgSO_4	39.9	47.7
NaCl	15.8	27.8	$(\text{NH}_4)_2\text{SO}_4$	1.4	1.2
MgCl_2	23.3	39.7	NaNO_3	12.4	2.5
CaCl_2	6.7	8.5	$\text{Ca}(\text{NO}_3)_2$	3.7	3.4
BaCl_2	0	0	KH_2PO_4	61.6	64.8
NH_4Cl	0.6	1.4	$(\text{NH}_4)_2\text{HPO}_4$	0	0
Distilled water		6.8			

* One molar van't Hoff solution contains the following salts in 115.8 liters of water: 100 M. NaCl , 2.2 M. KCl , 2.0 M. CaCl_2 , 3.8 M. MgSO_4 , 7.8 M. MgCl_2 .

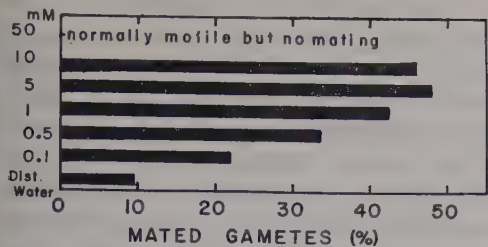


Fig. 3. Effect of concentrations of medium on induction of sexual activity (van't Hoff solution).

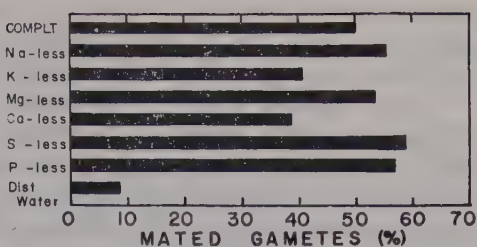


Fig. 4. Test of nutritional requirements for induction of sexuality (conditions of experiment, see text).

cell permeability, or for changing the physical state of protoplasm^{12,13}). The following experiment also supports this view.

Ionic interaction on induction of sexuality: The induction of sexuality was examined with incubation media of various ratios of KCl/CaCl₂, KCl/MgCl₂, or MgCl₂/CaCl₂. As shown in Fig. 5, the degree of induction of sexuality, as measured by the frequency of mated gametes, was always higher when two of the tested ions had been simultaneously present in the incubation medium, the best result being obtained in a mixture of KCl and CaCl₂ (5 mM.: 5/8 mM.), and also in the mixtures containing various ratios of KCl and MgCl₂. Although a similar increase in sexual induction was obtained in the mixtures of MgCl₂ and CaCl₂, the mixture was less effective in this respect than the other two combinations mentioned above.

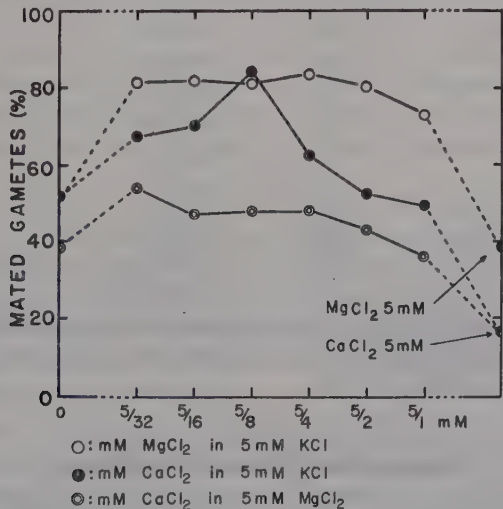


Fig. 5. Induction of sexuality as affected by the ionic interaction (conditions of experiment, see text).

Mating activity of sexually induced cells as affected by various salts: In the experiment described above, it was clearly shown that a high sexual activity was induced in cells incubated in the light in a solution containing ionic pair of mono- and bivalent cations in an adequately balanced ratio. To elucidate the possible effects of these ions on the subsequent step of sexual reproduction, i.e., mating activity, the follow-

ing experiments were performed. In view of the fact that the composition of the incubation media may probably have been considerably altered after a culturing period of 15 hrs. (see above), the mating test was carried out with washed gametes suspended in a fresh solution of given composition. For this purpose, growing cells of each mating type were washed and suspended in a solution containing KCl (5 mM.) and $MgCl_2$ (5/8 mM.), and incubated in the light for 15 hrs. After this treatment, the harvested cells were found to be sexually fully active. The gametes of each mating type were washed twice with distilled water and distributed into fresh salt solutions of 17 different kinds (Table 2). After 3 hr.-incubation in the light, the cells of the complementary mating types in each set of salt solution were mixed and kept in the dark. Counts were made for the mating pairs formed in 2.5 hrs. As shown in Table 2, the mating activity was found to be the highest in a solution containing both KCl and $MgCl_2$. The differences in effects of promoting and suppressing elements are much more clear-cut than those in the previous case of the induction of sexuality (Table 1). Here, the presence of bivalent cations with a single exception of Ba^{++} , promoted the mating, while monovalent cations such as K^+ , Na^+ , or NH_4^+ markedly suppressed the process. The suppressing effect of N-substances such as NH_4Cl , $(NH_4)_2SO_4$, NH_4NO_3 , $(NH_4)_2HPO_4$, $Ca(NO_3)_2$ and KNO_3 are also clearly demonstrated.

To recapitulate, Mg^{++} promotes both sexual differentiation and mating reaction, while Ba^{++} , Li^+ , NH_4^+ and NO_3^- inhibits both processes. Such factors as Na^+ and K^+ promote only the induction, while Ca^{++} only the mating process.

Table 2. Mating frequencies (%) of the sexually functional cells in inorganic salt solutions (5 mM. each)*

KCl and $MgCl_2^{**}$	72.5	Na_2SO_4	1.5
Distilled water	35.2	$(NH_4)_2SO_4$	0.5
LiCl	0.3	$MgSO_4$	66.3
KCl	0.6	NH_4NO_3	0.8
NaCl	1.5	$Ca(NO_3)_2$	26.8
MgC	34.9	KNO_3	0.3
$CaCl_2$	63.8	K_2HPO_4	0.5
$BaCl_2$	0.6	Na_2HPO_4	0.8
NH_4Cl	1.4	$(NH_4)_2HPO_4$	0.8

* Sexually functional cells of each mating-type were separately cultured in each solution, then mixed together and kept in the dark for further 2.5 hrs.

** KCl 5 mM. and $MgCl_2$ 5/8 mM.

Discussion and General Conclusion

One of the most clear facts reported in this study is the depression of sexuality in *C. moewuui* var. *rotunda* caused by the supply of assimilable N in the medium. The mating process *per se* is also found to be strongly affected by the same factor. Nutritional balance seems to make one of the possible mechanisms causing dedifferentiation of the gametic activity in the presence of assimilable N. In fact, when N was assimilated by the N-starved cells, synthesis of material for vegetative growth must recur, thus providing a physiological state unsuitable for mating. Sager and Granick⁷⁾ reported in their attempts to compare C/N ratio of the gametes with that of the vegetative cells in *C. reinhardi*, whose sexuality was also shown to be dependent on

the concentration of N in the medium, that the ratio did not correlate with the sexual activity of cells. They concluded that induction of sexuality was controlled not by the level of total N, but by the concentration of some specific N fraction or compounds in the cell.

According to Syrett¹⁴), when $(\text{NH}_4)_2\text{SO}_4$ was added to the culture of N-starved cells of *Chlorella vulgaris*, 75~80% of the added ammonium was assimilated, in the first 30 min., into the fraction of soluble N, and also into insoluble N, presumably chiefly protein. Although sexuality has not been reported in *Chlorella*, the circumstance concerning such assimilating processes must also be the case in *Chlamydomonas*. The question remains open for future investigation whether such sequence of events towards synthesis of cellular N-material prevents the formation (or induces the exhaustion) of sexual substances in the cell, and thus suppresses (or dedifferentiates) sexuality. Certain types of sexual substance have been demonstrated in *Chlamydomonas*^{1,9,15}).

On the other hand, it was also unambiguous that cells cultured in the ionic unbalanced medium could not perform their sexual reproduction. A combination of mono- and bivalent cations as K/Ca or K/Mg provided medium suitable for the occurrence of sexuality. The promoting effect of Ca^{++} or Mg^{++} on the mating reaction observed in the present organism was in agreement with that described by Lewin¹) in the type strain of *C. moewusii*. There have been numerous works reporting that the importance of Ca^{++} for the fertilization in various organisms¹⁷⁻¹⁹). Inasmuch as Ca^{++} or Mg^{++} is known to induce gelation at cell cortex¹²), such a physical alteration may take place, in the presence of these ions, at the flagella surface of *Chlamydomonas*; this will favor the contact and adhesion of cells at the tips of flagella in the mating. In contrast to the above mentioned activity of the bivalent cation, all the monovalent cations inhibit the mating reaction; NH_4^+ may not be an exception. However, the fact observed in the dedifferentiation-experiment would not have been due to the difference in the ionic activities between NH_4^+ and Na^+ , but due to the nutritional one. For, those media seem to be well balanced in the rest of other factors.

As observed in the experiments for induction of sexuality, this process preferred K^+ or Na^+ to Ca^{++} , i.e. the two processes in the sexual reproduction quite differed from each other in their ionic preference. Nevertheless, it was emphasized that none of those ions tested in the present study could hold the full sexual activity, and also that none of them was indispensable for the sexual reproduction; a physiological balance in the culture medium produced by the ionic interaction was an essential factor for the performance of sexuality.

The author wishes to express his gratitude to Prof. H. Hirose of Kobe University and to Prof. S. Imamura of Kyoto University for their encouragement during the work. He is also deeply indebted to Prof. A. Takamiya, University of Tokyo for the kind suggestion in preparing this paper.

Summary

Induction of sexuality in *Chlamydomonas moewusii* var. *rotunda*, which was used as *C. sp. 24* in the previous experiment, was dependent on the concentration of assimilable N in the medium (Tsubo, 1956²). The sexual activity of cells dedifferentiated also in the presence of assimilable N. Since it was supposed that the inhibition of mating reaction by N would be due not only to the nutritional, but also to the ionic interference, the experiments were performed to see the effect of each ion

used in the culture medium on the sexual reproduction of this alga. Bivalent cations such as Ca^{++} and Mg^{++} promoted, but monovalent cations such as K^+ or Na^+ inhibited the mating reaction. On the other hand, K^+ , Na^+ and Mg^{++} , but not Ca^{++} promoted the preceding step, induction of sexuality. NH_4^+ and NO_3^- inhibited both of these processes. Furthermore, it was noticed that none of these ions was indispensable, but a physiological balance in the medium produced by the ionic interference, e.g. with K^+ and Ca^{++} , or K^+ and Mg^{++} , was important for the accomplishment of sexual reproduction.

References

- 1) Tsubo, Y., J. Protozool. **8**: 114 (1961). 2) —, Bot. Mag. Tokyo **69**: 1 (1956). 3) —, Abstract in the 20th annual meeting of the Bot. Soc. Japan (1955). 4) Klebs, G., "Die Bedingungen der Fortpflanzung bei einigen Algen und Pilzen" G. Fischer, Jena (1896). 5) Czurda, V., Beih. Bot. Zbl. **51**: 711 (1933). 6) Nakano, H., Jour. Coll. Sci. Imp. Univ. Tokyo **40**: 1 (1917). 7) Sager, R. and Granick, S., J. Gen. Physiol. **37**: 729 (1954). 8) Trainer, F. R., Amer. J. Bot. **45**: 621 (1958). 9) Tsubo, Y., Bot. Mag. Tokyo **70**: 327 (1957). 10) Hutner, S. H., and Provasoli, L., in "Biochemistry and Physiology of Protozoa" ed. Lwoff, A. I: 27, Academic Press, N. Y. (1951). 11) Saito, K., "Hakko-Biseibutsu-Gaku" (in Japanese) 3rd imp. Maruzen, Tokyo (1948). 12) Heilbrumm, L. V., "An Outline of General Physiology" 3rd ed. W. B. Saunders Co., Philadelphia (1952). 13) Sakamura, T., J. Faculty Sci. Hokkaido Imp. Univ. 5th ser. **2**: 287 (1933). 14) Syrett, P. J., Annals Bot. N. S. **17**: 1 (1953). 15) Förster, H., and Wiese, L., Z. Naturforsch. **10 b**: 91 (1955). 16) Lewin, R. A., in "Sex in Microorganisms" ed. Wenrich D. H., A.A.A.S. Symp.: 100 (1954). 17) Hiwatashi, K., Sci. Rep. Tohoku Univ. 4th ser. **11**: 207 (1955). 18) Metz, C. B., in "Sex in Microorganisms" ed. Wenrich, D. H., A.A.A.S. Symp.: 284 (1954). 19) Rothschild, L., "Fertilization" Methuen, London (1956).

摘 要

坪 由 広: クラミドモナスの生殖に対する培地のイオン組成の影響について

Chlamydomonas moewusii Gerloff var. *rotunda* Tsubo において、配偶子形成が培地中の N 源の濃度に依存していることを、著者はすでに報告した。一方、N 源の存在するときには配偶子の接合能力がおさえられ、配偶子はふたたび栄養細胞になるものと思われた。接合の阻害機構としては、N 源のとりこみによる栄養的阻害が考えられるが、他方、接合現象が鞭毛の接触により始まるので、この表面現象に対する物質のイオンとしての阻害作用も考えられねばならない。そこで、本研究ではこの藻の有性生殖に対する培地のそれぞれのイオンの作用についてしらべてみた。その結果、 Ca^{++} や Mg^{++} のような 2 価の陽イオンは接合を促進し、 K^+ や Na^+ のような 1 価のものはこれを阻止した。逆に K^+ , Na^+ , Mg^{++} は前段階すなわち配偶子形成をうながし、これに対し Ca^{++} は逆の立場にあると考えられた。 NH_4^+ および NO_3^- は、ともに上記 2 つの生殖段階のいずれをも阻止した。さらに、本実験において試みられたイオンの中には、それらのうちどれかが生殖にとって必要不可欠であるというものはなかったが、この現象が遂行されるためには、培地中のイオン相互作用、たとえば K^+ と Ca^{++} , あるいは K^+ と Mg^{++} のような組み合わせによって生じる生理的平衡が重要な因子であることがわかった。(神戸大学理学部生物学教室)

球果の発育からみたスギ科の類縁関係 (追補) メタセコイアの球果の発育

肥 田 美 知 子*

Michiko HIDA*: The Comparative Study of Taxodiaceae from the Stand-point of the Development of the Cone Scale (Supplement)
The Development of *Metasequoia's* Cone Scale

1961 年 5 月 26 日受付

前に¹⁾ スギ科植物の類縁関係を球果の発育に伴う形態の変化からみてスギ科植物を5群に分け、他の形態からみた類縁関係と比較した。しかし、当時メタセコイアの球果に関しては、その内地での樹令が若く、開花数も少なく、発育までの各段階のものをみるができなかったのも、もっぱら雌花と成熟果の形態のみ、それにセコイアメスギでみた発育状態からメタセコイアの球果の発育を推定したにすぎなかった。今回大阪市立大学の高田英夫博士のご好意によりいろいろの発育段階の球果を手に入れることができ、また、成熟果は同大学の粉川昭平氏採集のものを観察させてもらったので、その発達の過程を一層明らかにすることができた。そこでここにその概要を報告する。

観察および考察

3月20日頃の球果は長径約4mm、短径約2mm、の小さな楕円体で、10mmばかりの花柄をもち、通常、枝の先端近くに側着しているが、まれに頂生することもある。本調査では95個体中、ただ1個体だけ頂生しているものがあった。花柄には5~9対の鱗片葉が着き、球花に近い2対は他より肉厚で、後に球果の形成にあずかる。球花はおおむね20片の鱗片からなり、そのうち先端の2~3対は胚珠を着けない。各鱗片は肉厚で表面観は菱形に近

い5ないし6角形をしている。縦断面では背軸側に大きな樹脂腔があり、その向軸側を管束が走り、その先は鱗片の突起部に達している。鱗片の向軸面はふくれあがり、ここに胚珠をつけ、主軸から分かれた他の一つの管束がこの着点まできている (Fig. 1, I の A, B, C)。

球果の成熟するにしたがい、長径より短径の伸びの方が大きく、長径:短径の値は小さくなり、球果は次第に丸味をおびてくる (Table 1)。各鱗片は向軸側の胚珠の着点から軸までの間がいちじるしくのび、それに伴って着点まできていた管束もしだいに外方までのびてくる。その上、鱗片は厚さを増す。したがって3月の球花の鱗片にみられた先端の突起部はそり返る。この後から発達してくる部分が実片で最初の鱗片の大部分は苞片と考えられる。球花の発達と同時に花柄も上部の2対の鱗片葉を残して下部の節間がのびる。そして上部の2対のうち、球果に近い1対は球果に接着する (Fig. 1, II の A, B, C)。

5月上旬になると球果は長径11mm、短径8.5mmぐらゐにまで発達し長径:短径の値はさらに小さく1.29の値を示す (Table 1)。各鱗片は向軸面において発達して外方に突き出し、かつそり返るので、苞片の先端は鱗片の中央部にまでおしやられ、鱗片を外側からみた形は口唇状になる。最下部の鱗片も他と同様にそり返る。他方、花柄の上部の2対の鱗片葉の着く部分のはびないので、最上部の1対の鱗片葉は球果のうちにはいりこむような恰好になる (Fig. 1, III の A, B, C)。

口唇状に十分発達した球果の鱗片のうち、上唇に

* Biological Laboratory of Osaka Women's University, Osaka, Japan. 大阪女子大学生物学教室

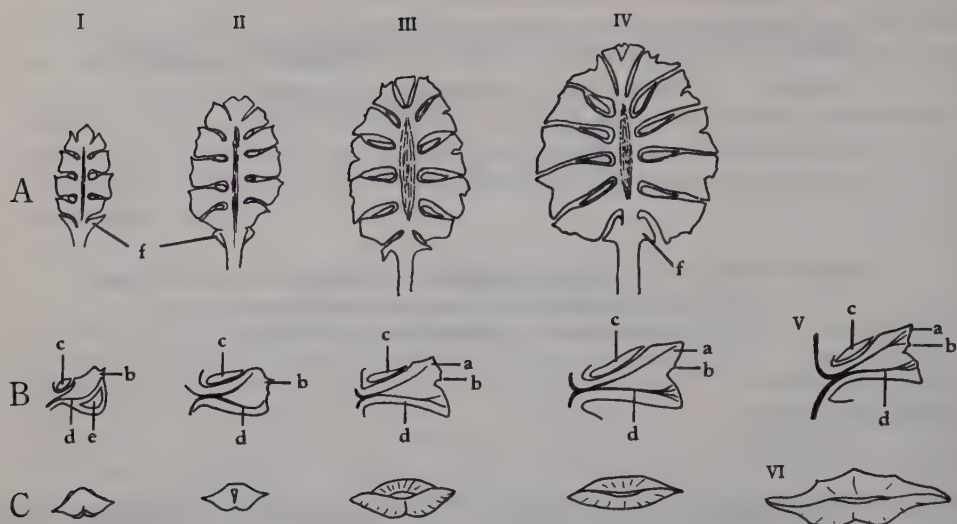


Fig. 1. The development of cone in *Metasequoia glyptostroboides*.

I, cone in March; II, cone in April; III, cone on May 5; IV, cone on May 15; V, cone in June; VI, cone in winter; A, longitudinal section of the cone; B, longitudinal section of the cone-scale; C, front view of the cone-scale; a, ovuliferous scale; b, bract; c, ovule; d, vascular bundle; e, resin cavity; f, cataphyll.

Table 1. The size of the female cone and the length of the peduncle in *Metasequoia glyptostroboides*.

Date of sampling	Long axis (mm.)			Short axis (mm.)			Long axis Short axis			Length of peduncle (mm.)		
	Max.	Min.	Av.	Max.	Min.	Av.	Max.	Min.	Av.	Max.	Min.	Av.
Mar. 20	5.0	4.0	4.5	2.5	2.0	2.25	2.0	2.0	2.0	12.0	10.0	11.0
Apr. 15	7.0	7.0	7.0	5.0	4.5	4.75	1.56	1.40	1.48			
May 5			11.0			8.5			1.29			
May 15			14.0			11.0			1.27			
Jun. 21	23.0	13.0	18.6	16.0	12.0	14.6	1.44	1.09	1.27	75.0	21.0	32.5
Jan.	36.0	10.0	20.2	21.0	9.0	15.4	1.71	1.00	1.28	90.0	15.0	12.8

相当する部分は後に発達してきた実片で、下唇は内側からの発達によって突き出してきた実片の外側に、もとの苞片部が接着したものである。はじめの苞片の突起は口唇状鱗片の中央部に小さな突起として残っている。鱗片の管束は Fig. 1 にみるように鱗片の表面近くで分岐して広がっている (Fig. 1, IV の A, B, C および V)。

球果が完全に發育すると乾燥し、各鱗片の間が開く。この時期の球果 80 個についてしらべた大きさは、Table 1 のようで、長径：短径の値が 1.1～

1.3 のものがもっとも多かった。果柄はよく伸長したものでは花の時の 8 倍に達しているが、なかにはほとんど伸びないものもあった。成熟果の鱗片は上部の 2～3 対を除いて全部大きな翼をもった種子を蔵しているが、まれに最下部の 1 対に種子をもたないものがあった (Fig. 1, IV)。果柄の最上部の 1 対の鱗片葉は多くの場合そり返った球果の鱗片におおわれるので、球果の下側に見られる鱗片葉の多くは 2 対目のものである (Fig. 2, C)。時には果柄の最上部の鱗片が球果内にはいりこまず、2 対の鱗片を

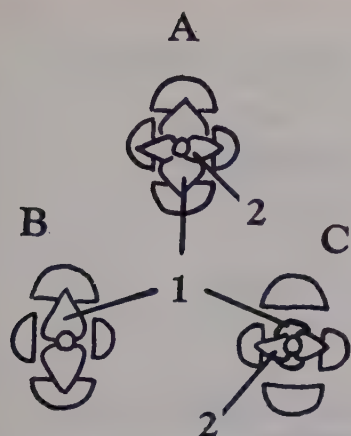


Fig. 2. Two pairs of cataphylls on the top part of the peduncle of *Metasequoia*'s mature cone (view from lower side).

A, with two pairs; B, the top pair only; C, top pair covered with the cone-scale and the next pair adhering to the lower cone-scale.

はっきり区別できる場合 (Fig. 2, A) や、2 対目が最上部のものよりやや離れている場合がある (Fig. 2, B).

以上の観察によって前報告¹⁾で推定したとおり、メタセコイアの球果はセコイアメスギやセコイアオスギの属する第4群、すなわち「雌花および球果のいずれにおいても実片、苞片の区別が明らかでなく、雌花の鱗片の大部分は苞片で、成熟に伴い実片がいちじるしく発達し、球果では苞片はまったく実片の付属物のような状態になるもの」にはいる。

このことは、球果の発達の過程からみてメタセコイアはスギ科内では他のものより、セコイアメスギ、セコイアオスギにちかいことを示すものである。

終わりに、終始ご親切なご教示を賜った大阪市立大学理学部の三木茂博士に厚くお礼申し上げるとともに、貴重な材料をご恵与下さった同大学の高田英夫博士ならびに粉川昭平氏に深く感謝する。

文 献

- 1) 肥田美知子, 植雑 70: 44 (1957).

Summary

When I reported about the morphology of female cones of Taxodiaceae, *Metasequoia* trees in Japan were so young that I could not get cones of various maturity. However, last year Dr. Hideo Takada kindly gave me these cones, and I made observation on them. The result is that the development of female cones of *Metasequoia* was the same as in the case with *Sequoia sempervirens* (Fig. 1), i.e. the bract and the ovuliferous scale can not be distinguished from each other on the female cone. The ovuliferous scale develops remarkably during mature time of the cone. The form of mature cone-scale is bilabial, whose upper lip implies the ovuliferous scale and lower lip consists of ovuliferous scale and bract. The ovuliferous scale and bract can not be distinguished from each other externally in the adult cone. These results suggested that *Metasequoia* is more intimately related to *Sequoia* and *Sequoiadendron* than to other members of Taxodiaceae.

The top and the next pairs of cataphylls on the peduncle which showed no elongation of the internode attach to the base of the mature cone (Fig. 2, A). Mostly the top pair is covered with the recurved lower cone-scale (Fig. 2, C) and occasionally only the top pair adheres to the cone (Fig. 2, B).

Micrococcus glutamicus の細胞学的研究

第5報 クエン酸ナトリウムおよび

リンゴ酸ナトリウムの伸長肥大

分岐効果について*

板垣史郎**・木幡 守**・木下祝郎**

Shiro ITAGAKI**, Mamoru KOBATA**, and Shukuo KINOSHITA**: Cytological Studies on *Micrococcus glutamicus*
Part V. The Effects of Na-citrate and Na-malate on Cell Elongation and Branching

1961 年 6 月 14 日受付

緒 言

Micrococcus glutamicus の合成培地中の形態は、その必須成分たるビオチンによりほとんど決定的な影響を受けることはすでにくり返し報告した¹⁻³⁾。

著者らは、ビオチン以外の物質、あるいは物理的条件などで形態に大きな影響を与える因子について検討を進めてきたが、クエン酸ナトリウムおよびリンゴ酸ナトリウムの存在下で、本菌がいちじるしい長大化と、分岐をおこなうことを見いだした。

以下、これらの条件、分岐細胞の細胞学的構造について報告する。

実験材料および実験方法

使用菌株: *Micrococcus glutamicus* をはじめとするグルタミン酸生産菌 9 菌株を用いた。

使用培地および培養方法: glucose bouillon で前培養をおこない、これをクエン酸ナトリウムまたはリンゴ酸ナトリウムを添加した合成培地に 10% の割に加え、rotary shaker にて 28° で培養した。

* 第 25 回植物学会大会にて発表

** Tokyo Research Laboratory, KYOWA Fermentation Industry Co., Ltd., Tokyo, Japan.
協和醸酵工業株式会社東京研究所

培養の進行にしたがい、適宜培養液を採取し、pH の変化、菌体増殖を測定、遠沈集菌により顕微鏡試料を作製した。

各試料につき、Löffler のアルカリ性メチレン青染色、Webb⁴⁾ の方法を一部改変した細胞膜染色、塩酸ギムザの方法を一部改変した核染色をほどこした。

結 果

1. GPM 培地にクエン酸ナトリウムおよびリンゴ酸ナトリウムを添加した場合

既報のごとく、*M. glutamicus* は、glucose bouillon (GPM) 中では整一な類楕円形を呈するが、このような有機酸を多量に添加することにより多少菌形態は不整化する傾向にあるが、その程度は小さい。

2. 合成培地にクエン酸ナトリウムおよびリンゴ酸ナトリウムを添加した場合

ビオチンを含有 (ビオチン量 1 γ /l および 100 γ /l) する合成培地にクエン酸ナトリウムおよびリンゴ酸ナトリウムを 1, 5 および 10% 添加し、glucose bouillon 前培養を 10% 添加した。はじめ、*M. glutamicus* 560 についてのみおこなったところ、5 および 10% 添加においていちじるしい

第1表 クエン酸ナトリウムおよびリンゴ酸ナトリウム添加による生育と分岐形成の関係

Strain	Biotin content r/l	Acid		Branching			Growth (O.D.)		
				24h	48h	72h	24h	48h	72h
M. 516	1	Na-malate	5%	—	—	—	0.465	0.570	0.520
			10%	+	+	+	0.033	0.045	0.046
		Na-citrate	5%	+	+	+	0.152	0.183	0.165
			10%	+	+	+	0.132	0.083	0.072
	100	Na-malate	5%	—	—	—	0.265	0.292	0.282
			10%	+	+	+	0.104	0.133	0.116
		Na-citrate	5%	+	+	+	0.155	0.173	0.155
			10%	+	+	+	0.136	0.130	0.106
M. 534	1	Na-malate	5%	—	—	—	0.415	0.400	0.380
			10%	+	+	+	0.033	0.035	0.040
		Na-citrate	5%	+	+	+	0.060	0.065	0.081
			10%	+	+	+	0.020	0.023	0.027
	100	Na-malate	5%	+	+	+	0.224	0.242	0.232
			10%	+	+	+	0.115	0.124	0.120
		Na-citrate	5%	+	+	+	0.075	0.072	0.086
			10%	+	+	+	0.062	0.048	0.052
M. 541	1	Na-malate	5%	—	—	—	0.432	0.390	0.375
			10%	+	+	+	0.032	0.033	0.046
		Na-citrate	5%	+	+	+	0.056	0.065	0.063
			10%	+	+	+	0.025	0.023	0.030
	100	Na-malate	5%	±	±	±	0.228	0.265	0.235
			10%	+	+	+	0.105	0.114	0.120
		Na-citrate	5%	+	+	+	0.083	0.113	0.146
			10%	+	+	+	0.050	0.055	0.055
M. 560	1	Na-malate	5%	—	—	—	0.403	0.355	0.340
			10%	+	+	+	0.068	0.067	0.046
		Na-citrate	5%	+	+	+	0.193	0.190	0.180
			10%	+	+	+	0.115	0.106	0.094
	100	Na-malate	5%	—	—	—	0.222	0.240	2.212
			10%	±	±	±	0.162	0.190	1.165
		Na-citrate	5%	±	±	±	0.155	0.205	1.177
			10%	±	±	±	0.095	0.110	0.090
M. 582	1	Na-malate	5%	—	—	—	0.395	0.485	0.525
			10%	+	+	+	0.034	0.028	0.050
		Na-citrate	5%	+	+	+	0.047	0.055	0.052
			10%	+	+	+	0.068	0.060	0.070
	100	Na-malate	5%	—	—	—	0.203	0.255	0.267
			10%	—	—	±	0.125	0.145	0.150
		Na-citrate	5%	±	±	±	0.127	0.153	0.157
			10%	+	+	+	0.090	0.114	0.115
M. 588	1	Na-malate	5%	—	—	—	0.398	0.345	0.325
			10%	+	+	+	0.035	0.032	0.034
		Na-citrate	5%	+	+	+	0.141	0.100	0.102
			10%	+	+	+	0.022	0.005	0.010
	100	Na-malate	5%	—	—	—	0.185	0.180	0.197
			10%	—	—	—	0.130	0.115	0.098
		Na-citrate	5%	—	—	±	0.145	0.140	0.118
			10%	—	—	—	0.030	0.015	0.017

第1表 つづき

Strain	Biotin content r/l	Acid	Branching			Growth (O. D.)		
			24h	48h	72h	24h	48h	72h
M-7001	1	Na-malate	5%	—	—	0.403	0.376	0.360
			10%	+	+	0.100	0.085	0.035
		Na-citrate	5%	+	+	0.225	0.200	0.153
			10%	+	+	0.125	0.128	0.100
	100	Na-malate	5%	—	—	0.194	0.220	0.202
			10%	±	—	0.168	0.206	0.186
		Na-citrate	5%	—	±	0.175	0.175	0.150
			10%	—	+	0.144	0.137	0.121
S-1627	1	Na-malate	5%	—	—	0.375	0.360	0.375
			10%	±	±	0.217	0.271	0.241
		Na-citrate	5%	+	+	0.168	0.195	0.164
			10%	±	±	0.072	0.098	0.042
	100	Na-malate	5%	+	+	0.264	0.293	0.265
			10%	+	±	0.152	0.200	0.186
		Na-citrate	5%	—	±	0.138	0.178	0.150
			10%	±	±	0.061	0.090	0.078
S-1	1	Na-malate	5%	—	—	0.435	0.420	0.350
			10%	+	+	0.042	0.073	0.030
		Na-citrate	5%	+	±	0.025	0.040	0.026
			10%	+	+	0.120	0.063	0.025
	100	Na-malate	5%	—	—	0.238	0.281	0.245
			10%	—	±	0.140	0.190	0.137
		Na-citrate	5%	+	+	0.115	0.187	0.147
			10%	+	+	0.090	0.106	0.064

菌の伸長不整化と、分岐細胞の混在をみた。

グルタミン酸生産菌9菌株における、生育と分岐形成の関係の1例を一括して第1表に示した。この表で注意を要することは、分岐形成の場合はいずれも極度に生育不良であることである。

生存する細胞はいちじるしい伸長肥大形を呈することから、接種菌体の大部分はおそらく溶菌死滅しているものであろう。

リンゴ酸ナトリウムを添加した場合、分岐は二次的にもおこることがあるが、リンゴ酸ナトリウム添加培地での溶菌はきわめていちじるしく、生き残る菌体は溶菌塊に埋もれてはっきりとした形態を観察することは困難である。

3. 分岐形成の時期

(1) クエン酸ナトリウム添加の場合

ビオチン r/l	クエン酸ナトリウム (%)	分岐の認められた時間
1, 100	5, 10	24 時間以降

(2) リンゴ酸ナトリウム添加の場合

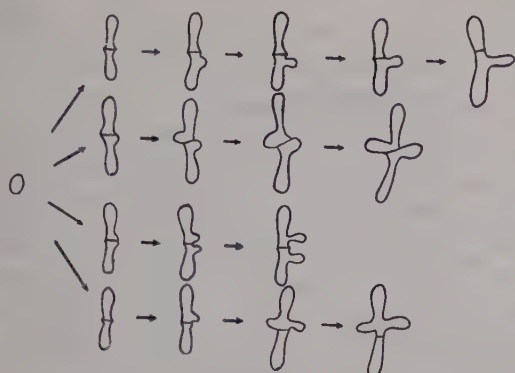
ビオチン r/l	リンゴ酸ナトリウム (%)	分岐の認められた時間
1	10	12 時間以降
100	5, 10	24 時間以降

4. 分岐の形成

(1) クエン酸ナトリウム添加の場合

クエン酸ナトリウム5%添加で細胞は特に肥大、伸長し、末端細胞はいちじるしく膨大、典型的なこん棒状を呈する。分岐の形成は1個のみである場合が多い。まれには十字型の細胞が認められることもある。

クエン酸ナトリウムの添加量を10%に増加すると、菌はいずれもいわゆる bifid bacteria にみられるごとき形態を呈するに至る。この形態は、逆に考えると、分岐形成の第一歩とも考えられる。すなわち、写真6~13および第1図にしめすごとく、中央部に接する二つの細胞はそれぞれその点よりこぶ状の突出をつくり、漸次伸長して短い分岐を形成する。しかし、環境不良のためその点で生育は停止せしめられる。クエン酸ナトリウムが5%程度の場



第1図 Bifid bacteria より分岐の形成

合は伸長がさらに続行するために明瞭な分岐として認められるに至る。

(2) リンゴ酸ナトリウム添加の場合

大部分の菌は溶菌をおこすが、生き残った菌は伸長し、1~数個の分岐が形成される。まれながら、分岐にさらに二次的に分岐が形成される場合もみられる。リンゴ酸ナトリウム添加培地では菌は12時間位ですでに溶菌をおこし視野は不鮮明となってくる。溶菌による溶菌塊中に分岐細胞が認められる。

はじめに添加された接種菌体は、glucose bouillon中に生育したものであるから、当然菌形は類楕円形を呈しているが、リンゴ酸ナトリウム添加合成培地で培養を続け、O.D. (光学密度) 値により生育度を測定すると、むしろ減少している場合すらあるにもかかわらず、このように伸長肥大した菌が認められることは、大部分の菌体が生育できず、溶菌をおこしているものと解釈しえよう (第1表参照)。

5. クエン酸ナトリウムおよびリンゴ酸ナトリウム添加量と形態変化の関係

(1) クエン酸ナトリウム添加の場合

a) ビオチン 1 γ /l 添加培地

クエン酸ナトリウム添加 1~3% まで比例的に菌は伸長肥大、多細胞体を形成する。3% ではまれに分岐細胞が形成される。4, 5% では長大化の傾向は逆にやや減少するが、混在して認められる分岐細胞数は増加する。6% 以上添加すると、菌体は漸次短かくなり、いわゆる bifid bacteria 型⁵⁾を呈し、その傾向はクエン酸ナトリウム添加量の増大にともなう。O.D. 値は 1~3% まで漸次減少し、4% で急激な低下を示し、10% に至る。

b) ビオチン 100 γ /l 添加培地

クエン酸ナトリウム 2% 添加まで多細胞体とはならず、1ないし2細胞の状態であるが、3% より急激に長大化し、分岐が形成される。5% 以上添加の場合より菌は短かくなり、bifid bacteria 型が形成される。以下 10% 添加までその形態は変わらない。

O.D. 値は長大化と期を一にし、3% 添加より急激に減少する。

(2) リンゴ酸ナトリウム添加の場合

a) ビオチン 1 γ /l 添加培地

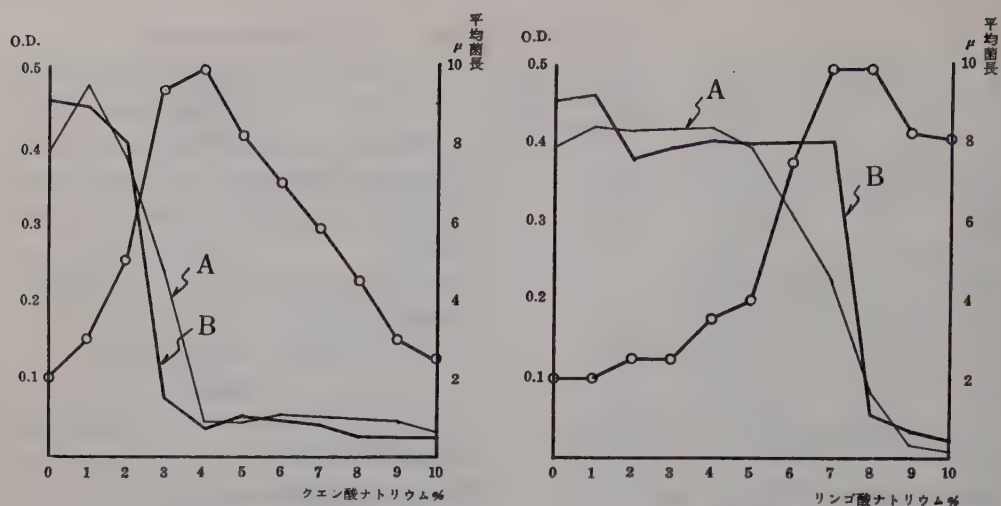
リンゴ酸ナトリウム 1~6% 添加までいずれも多細胞体を形成する。リンゴ酸ナトリウムの量の増加に比例して多細胞体の程度は増大し、長大化の傾向は明瞭である。一方、O.D. 値はこれと反対に漸次低下し、生育の不良化を示す。7% で分岐の形成が認められはじめ、長大菌中に分岐細胞の混在が認められる。8~9% より急に溶菌をおこし、溶菌塊中に長大菌、分岐細胞の混在が認められ、O.D. 値はいちじるしく低下する。

b) ビオチン 100 γ /l 添加培地

リンゴ酸ナトリウム 7% 添加までは良い生育を示し、形態も2細胞程度が大部分であり、ビオチン 1 γ /l の場合のごとく多細胞体ではない (ただし、6% 以上添加したものは培養2日以降多細胞体となる)。8% 添加で急激な溶菌が認められ、O.D. 値は7% のその 1/10 以下となる。この時溶菌塊中に長大菌と分岐細胞の混在が認められる。以下 10%

第2表 クエン酸ナトリウムおよびリンゴ酸ナトリウム添加量と菌長

添加量 %	クエン酸 ナトリウム	リンゴ酸 ナトリウム
0	2 μ	2 μ
1	3	2
2	5	2.5
3	9.5	2.7
4	10	3.5
5	8	4
6	7	7.5
7	6	10
8	4.5	10
9	3	8.2
10	2.5	8



第2図 クエン酸ナトリウムおよびリンゴ酸ナトリウム添加量と生育および菌長との関係 (2日培養)。

- 1) O.D. 値は培養液を 10 倍希釈して測定した。
- 2) 平均菌長は混在する小型菌は除外して測定した。
- 3) A: ビオチン 1 γ /l 培地, B: ビオチン 100 γ /l 培地。
- 4) ○—○: Aにおける平均菌長。

添加まではほぼ同所見である。

このように、リンゴ酸ナトリウム添加の場合は、クエン酸ナトリウム多量添加の場合にみられるとき *bifid bacteria* 型を認めない。

かくのごとく、生育の度合と形態の変化はそれぞれの酸の添加量と密接な関係を示す。この変化を第2表および第2図に示した。

6. 伸長肥大細胞および分岐細胞がもとの菌体 (inoculum) の変形であることについて

クエン酸ナトリウムあるいはリンゴ酸ナトリウムを上述のごとく添加する時は、必ず伸長、分岐、あるいは *bifid bacteria* 型 (クエン酸ナトリウム多量添加の場合) が形成される。この事実、逆にこのような培養条件下でのみ生育しうるある種の *contaminant* が発現してきたとも考えられぬこともない。この点を明確にするため、伸長、分岐細胞の移植実験を行なった。

すなわち、ビオチン 1 γ /l, クエン酸ナトリウム 5% 添加培地に *M. glutamicus* 582 を培養し、伸長、分岐細胞を形成させた後、これをビオチン 100 γ /l, クエン酸ナトリウム 0, 5, 10% 添加培地に移植した。すると、*glucose bouillon* 前培養をこれらの培地に添加した場合と同様の形態を示すに至

た。すなわちビオチン 100 γ /l, クエン酸ナトリウム 0% 培地においては、添加した伸長および分岐細胞はそれぞれ分裂し、ほぼ整一な類楕円形菌体を呈するに至った。またビオチン 1 γ /l の培地に添加した場合、ペーパー・クロマトグラフィーにより、培地中に 10~15 mg/ml のグルタミン酸を生成したことを認めた。これらのことより、伸長、分岐細胞は添加された菌体そのものの形態変化と考えてよいであろう。決定的な証明は単個菌培養による形態変化の追及であろうが、この点は目下検討中である。

7. Chelating agent の影響

クエン酸およびリンゴ酸はともに *chelating effect* をもっているため、これらの添加によるグルタミン酸生産菌の伸長、分岐のごとき形態変化は、この *chelating effect* による可能性がある。そこで EDTA 添加実験を試みた。

ビオチン 1 γ , 100 γ /l 添加合成培地 100 ml に対し、EDTA を 10^{-1} ~ 10^{-6} mol 添加し、*glucose bouillon* 前培養を加え、その生育、形態変化を追跡した。 10^{-1} ~ 10^{-3} mol 添加では生育はまったく認められず、形態的にはほぼ前培養のそれを保った。 10^{-4} ~ 10^{-6} mol 添加の場合は、EDTA 無添加の合成培地におけるとほぼ同一の生育、形態をしめし

た。

すなわち、EDTA の chelating effect は、本菌の形態変化には一応本質的な作用をもたないと言えよう。もちろん、このことをもってクエン酸ナトリウムあるいはリンゴ酸ナトリウムの chelating effect を一概に否定することはできないが、伸長、分岐などの変化は、クエン酸ナトリウムあるいはリンゴ酸ナトリウムの生理的効果と考えた方が妥当ではないだろうか。

8. 分岐細胞の構造について

(1) Septa の形成

ビオチン含量が少ない合成培地においては、本菌が多細胞体を形成し、ビオチンが多量に存在する時はほぼ単細胞の状態で生育することは既報のごとくであるが、分岐を形成するほどクエン酸ナトリウムあるいはリンゴ酸ナトリウムを添加した場合はビオチン 100 γ /l のごとく多量添加しても多細胞体となる。このことは細胞膜染色をほどこすことにより明らかである。このような染色標本をもちいて多数の分岐細胞を観察することから、分岐の形成過程が推定される。すなわち、いったん多細胞体を形成したのち、多細胞体の中の中間細胞の成長点が側方へ移動し、分岐として突出してくる。このようにして形成された分岐は、ある程度伸長すると、その親細胞との間に隔壁を形成し、さらに伸長を続け分岐の方も多細胞（多くの場合 2～3 細胞）により形づくられることになる。

しかし、クエン酸ナトリウムを多量に添加した場合は多細胞体とはならず、2 細胞の bifid bacteria 型が大部分である。この形の細胞は、2 細胞の接する点（隔壁の所）より、どちらかの細胞、あるいは両方の細胞（この場合十字形になる）がこぶ状に短い分岐を出す。その後やや伸長する場合もあるが、分岐に隔壁が形成されることはまれである。この進行のありさまを第 1 図に示した。

(2) 核構造について

分岐を形成するような培養条件は、いずれにしてもかなり異常な条件であるから、その菌体の生育、分裂様式にも大きな影響を与えるであろうことは想像に難くない。したがって、その外部形態のみならず、その内部構造、特に核構造、あるいは核分裂に与える影響も当然大きいであろう。このような見地より分岐細胞あるいは伸長細胞の核構造を観察し

た。

a) ラセン核

クエン酸ナトリウム添加培地で生じた伸長菌体において特記すべき形態としては写真 20, 21 にしめすごときらせん状の核が認められることである。このような形の核は *Spirillum* においては普通にみとめられるものであるが、本菌においては従来認められなかったもので、明らかにクエン酸ナトリウム添加の影響であると考えられる。この核については後報において詳細にのべる予定であるから、ここでは記載するにとどめる。

b) 点状核

比較的まれではあるが、小さい点状の核が細胞中にやや不規則に並んでいる場合が認められる。このものは、1 細胞あたり 1 個存在するものか、あるいは 1 細胞中に 2 個以上存在するものかは不明である。細胞の分裂が伴わないで核のみが分裂したものである可能性もあるが、これらの点は判然としない（写真 22）。

c) 巨大核

伸長細胞、あるいは分岐細胞においては一般に末端細胞は特に大きく、このために菌外形はこん棒状を呈する。この末端細胞中に存在する核は特に大きい塊状を呈するのが普通である。しかし、まれには菌体全体に特に大きい核が並んで存在する場合も認められる（写真 23）。

d) 分岐細胞の核

分岐は中間にはさまれた細胞より伸長形成されるであろうことは述べた。したがって、この細胞内の核が分裂して分岐中にはいっていくことは当然である。一般にクエン酸ナトリウム 5%, リンゴ酸ナトリウム 10% 添加のごとき、分岐がかなり伸長する条件下では、分岐の核も、もとの細胞におけると同様に無糸分裂により分裂していくものと考えられる。ただし、まれには分岐を形成した細胞の核が分裂せずにそのまま分岐中に移行し、そのためにその細胞が無核になってしまう場合も認められる（写真 25～27）。

e) 対称核

本菌の分岐形成の進行は、まず形態的にも、本質的にも bifid bacteria 型が先駆となると考えられる。Bifid bacteria 型は第 1 図にも示したごとく、中間の隔壁よりみて対称形をなす。したがって、本

菌が *bifid bacteria* 型よりさらに伸長したとき状態でも往々にして対称形の核が観察されることがある (写真 24)。

考 察

1. 伸長細胞,あるいは分岐細胞は contaminant ではないことの証明

クエン酸ナトリウムあるいはリンゴ酸ナトリウムを添加することによって形成された伸長細胞,さらには分岐細胞などの形態の細胞が contaminant ではなく,接種菌体の形態変化であることの証明は重要である。その証明の1として,これらの酸を加えないかぎりこのような形態を生ぜず,加えた場合は必ず生じる。しかし,この点は裏を返せば,そのような条件下でのみ生育しうるある種の菌が混入してきた結果であって,このような培地では本来の菌は生育しないと考えることも可能ではある。しかし,添加酸量の変化に応じた生育度合,菌形態変化などがみられることから,上述のごとく contaminant と考えることは妥当ではない。証明の2としては,酸添加培地で形成された長大細胞を酸無添加培地に移すことにより,従来述べてきたようなそれぞれの培地に特有な形態に還元をすることがあげられる。さらに第3として,伸長細胞もグルタミン酸生成能を保持していること,などがあげられる。

しかし,以上述べたとき証明は,菌体を集団として取り扱った結果であるから,決定的な証明とはなりえない。このためには,単個菌培養により個々の細胞の性質を明確にする必要があろう。その予備的観察の結果を次に述べる。

クエン酸ナトリウム添加培地で形成した分岐細胞を GPM 培地にてスライド培養し,観察を続けると,ある程度さらに伸長を続け,やがて分裂を開始する。この分裂の仕方はかなり複雑のようで,端の細胞から切れはじめるというわけでもなく,ばらばらに分裂をはじめるようである。かくして生じる多数の細胞は球ないし楕円形であるが,大小はまちまちである。この点についてはさらに詳細な検討を続けているので別に報告したい。

以上のごとき諸点より,本報において著者らが述べた伸長細胞,あるいは分岐細胞は,クエン酸ナトリウムあるいはリンゴ酸ナトリウムによりひきおこされた *M. glutamicus* をはじめとするグルタミン

酸生産菌の変形のひとつと考えてよいであろう。

2. 生育と分岐

細胞の長大化,分岐などが起こるようにクエン酸ナトリウムあるいはリンゴ酸ナトリウムを添加するときは,培地中に存在する菌の集団としての生育度合は一般に極度に不良であり, O.D. 値は非常に低い。にもかかわらず,長大化した細胞は時に幅 1.8 μ , 長さ 15 μ 以上にも達することがあることは,接種菌体の大部分が死滅溶菌していることを示すものといえよう。かくのごとき条件に生き残りをえた菌も元来生育に適した培地に移すことにより,それぞれに応じた生育と形態を示す。このことから酸添加により生じるこのような形態異常は一過的なものであると考えうる。

3. 分岐形成

分岐細胞はすでに述べたように多細胞体よりなる。多細胞体の中間細胞が,その生長点を変えて,側方より分岐として突出するものと考えられるが,中間細胞は数個,時には十数個にもおよぶにもかかわらず,形成される分岐は通常1個のみであること(特にクエン酸ナトリウム添加の場合)は重要である。また,どうして生長点が側方に変化するかのも不明である。ただ多細胞体の隔壁は正常分裂の途次に形成される隔壁とはやや性質を異にする点に関係するものと考えられる。すなわち,正常分裂の際に形成される隔壁はその部位よりただちに分裂,分離することが本質的な性質であるが,ビオチン欠乏状態などで形成された多細胞体の隔壁は,容易には分離せず,かなり強固に細胞同志をつないでいる。したがって酸添加で形成された多細胞体の隔壁もこのような強固なものであって容易には分離しないため,その点に存在するべき生長点は側方へ移行することもありうると考えられる。この考え方は第1図に示した *bifid bacteria* 型の形成からもある程度裏書きされると思われる。

要 旨

Micrococcus glutamicus をはじめとするグルタミン酸生産菌の形態変化につき検討し,合成培地中にクエン酸ナトリウムあるいはリンゴ酸ナトリウムを添加することにより,いちじるしい菌体の肥大伸長と分岐の形成をみた。

1. クエン酸ナトリウム添加
3% 添加より分岐の形成が認められた。4~5%に増加するとさらに分岐形成は明瞭となる。6% 以上にクエン酸ナトリウムの添加量を増加すると菌体は短くなり、いわゆる bifid bacteria 型を呈するに至る。

2. リンゴ酸ナトリウム添加
7% 以上添加することにより分岐が形成される。しかし、この場合大部分の菌は溶菌をおこす。リンゴ酸ナトリウムの添加では bifid bacteria 型の形成

は認められない。

このように伸長した肥大細胞は多細胞体であり、ある程度伸長した分岐もまた数細胞より形成されている。

種々ご指導、ご助言をいただいた東大教授湯浅明博士、東大教授北原覚雄博士に深く感謝いたします。また、実験にご協力くださった当所古川稔所員に感謝致します。

文 献

- 1) 板垣史郎・木下祝郎, 植雑 72: 51 (1959). 2) 板垣史郎, 植雑 73: 318 (1960). 3) 板垣史郎・古川 稔・木下祝郎, 東大応微研シンポジウム 第1集: 146 (1960). 4) Webb, R. B., J. Bact. 67: 252 (1954). 5) Sundman, V., Björkstén, K. A., Gyllenberg, H. G., J. Gen. Microbiol. 21: 371 (1959).

Summary

Observation of morphological changes was made in *Micrococcus glutamicus* and other glutamic acid-producing organisms cultivated in the synthetic media containing Na-citrate or Na-malate.

Elongated and branched cells were formed under such condition.

1. Addition of Na-citrate

In a medium containing 3% of Na-citrate, formation of branched cells was rarely observed. And in the medium containing 4 to 5% of Na-citrate, branching of cells appeared markedly. However, shortening of branches and simultaneous change of cell shape into so-called "bifid bacteria form" occurred when the concentration of Na-citrate increased up to 6% or more.

2. Addition of Na-malate

Branches were formed when cells were cultivated in the synthetic medium containing 7% or more of Na-malate. In this case, however, majority of cells were lysed.

"Bifid bacteria form" was not observed in the Na-malate medium.

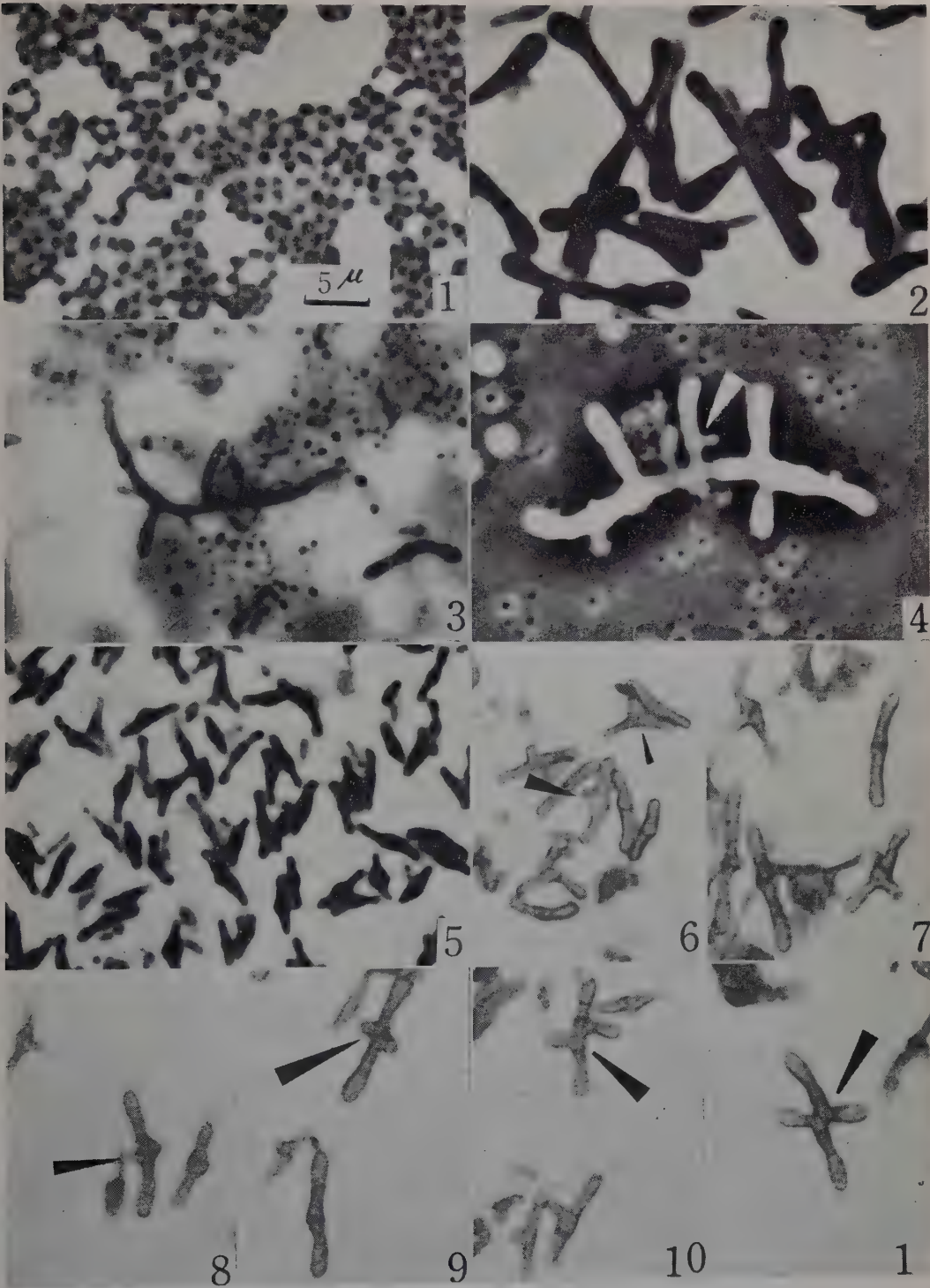
By Webb's cell wall staining method, it was observed that elongated or branched cells were always of multicellular form with several or more septa.

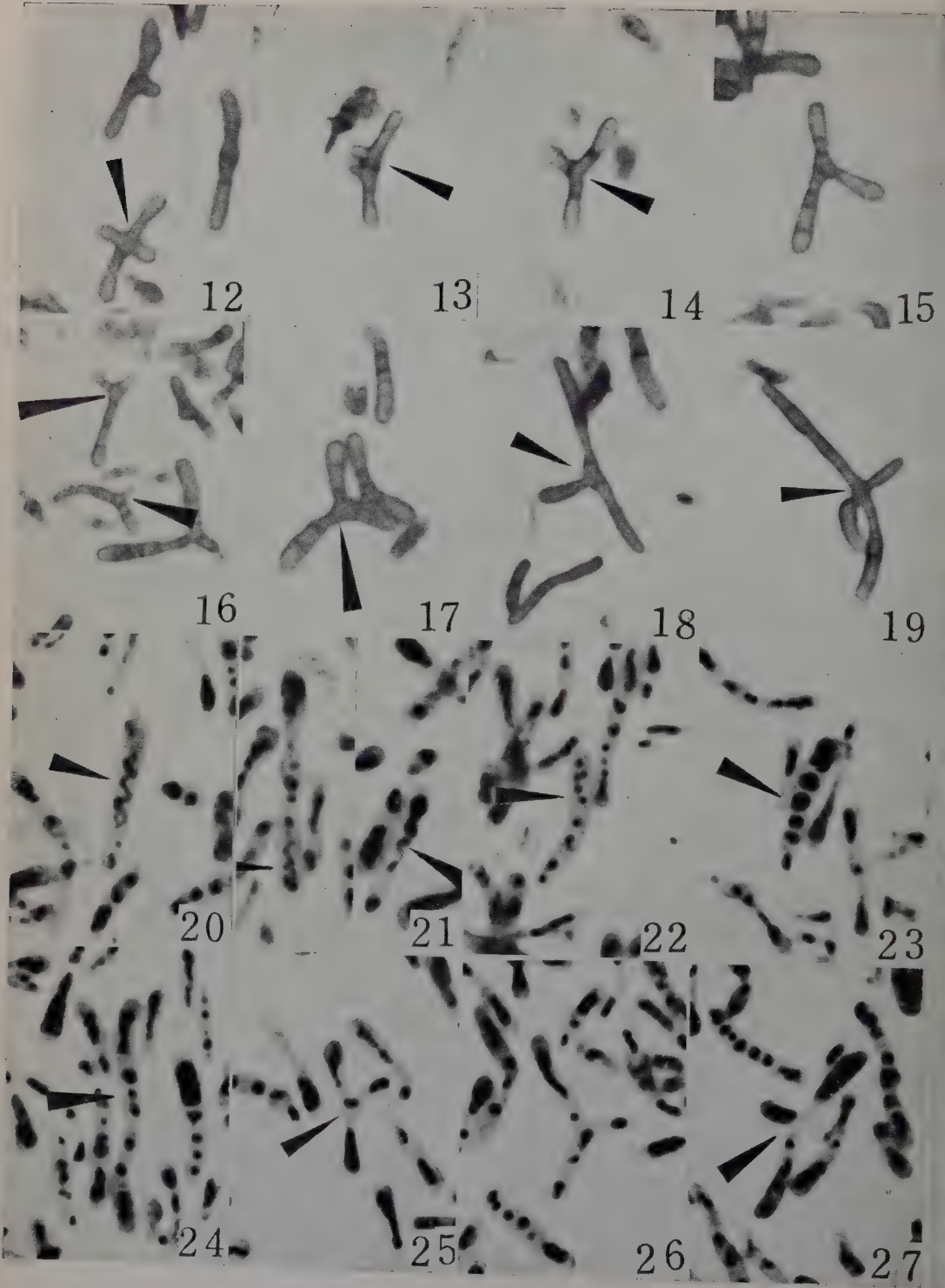
Spiral, large, point-like, and symmetrical nuclei were observed in elongated cells by HCl-Giemsa nuclear staining method.

As the branching of a cell proceeded, the nucleus of the cell was divided into two daughter nuclei, then, one of which moved into the branch.

写真説明

1. M. 560, glucose bouillon 培養, 類橢円形で齊一である。
2. M. 582, クエン酸ナトリウム 5%。
3. M. 560, リンゴ酸ナトリウム 10%。いちじるしい溶菌のため, 視野は鮮明さを欠く。溶菌塊中に数個の分岐をもった伸長細胞が認められる。
4. 同上, 位相差顕微鏡写真。二次分岐に注意。
5. M. 560, クエン酸ナトリウム 10% 添加, いわゆる *bifid bacteria* 型を呈する。
6. M. 516, クエン酸ナトリウム 10%。 *bifid bacteria* 型の短い分岐がみられる。
- 7~13. M. 582, クエン酸ナトリウム 10%。 *bifid bacteria* 型細胞よりの分岐をしめす。短い分岐からやや伸長したものまで。時に十字形, もしくは同じ側へ2本出ている場合もある。
- 14~17. M. 582, クエン酸ナトリウム 5%, Y字型分岐。やや伸長したものには隔壁が認められることより, 分岐は親細胞とも多細胞よりなることが知られる。
- 18, 19. S-1, クエン酸ナトリウム 5%。T字型および十字形の分岐をしめす。
- 20~27. M. 582, クエン酸ナトリウム 5%。塩酸ギムザ染色による核形態。
20. ラセン核をしめす。クエン酸ナトリウム 5% 添加培地中で, このようなラセン状を呈する核が往々にして認められる。
21. 末端の大型核に融合したセラン核。
22. 点状核。
23. 大型核。通常, 末端の核は大きい, 時にこのように大型核が菌体中に並ぶこともある。
24. 短い *bifid bacteria* 型の菌においては, その菌形のごとく, 核も対称形であるが, ここには伸長した菌体でみられる対称核をしめした。
25. 分岐細胞の核。分岐の根元に核が存在する。
26. 伸長した分岐中に明瞭な核が認められる。
27. 分岐の核は, その親細胞の核が分裂してはいっていくのが普通であるが, 時に分裂せず, そのまま分岐の方へ移行してしまう場合がある。このような時には分岐の根元は無核の状態になる。





Penicillium islandicum Sopp. の紫外線照射株における色素生産の消長

菊池正彦*・中原正城*

Masahiko KIKUCHI* and Masaki NAKAHARA*: Sequence of Pigment Formation in the Mycelia of Defective Strains Obtained from *Penicillium islandicum* Sopp. by UV-irradiation

1961 年 7 月 15 日受付

著者ら¹⁻³⁾は、さきに *Penicillium islandicum* Sopp. の発育途上における菌体色素の消長を調べることによって、アントラキノン系色素相互間の生成的連関を研究し、柴田の仮説⁴⁾が、一部の修正を除けば、ほぼ妥当であるとの結論に達した。

本報では、紫外線照射によって得られた菌株における色素生産の知見を報告し、あわせて菌体色素の生成的連関について考察したい。

材料および方法

菌株： この実験には *Penicillium islandicum* Sopp. NRRL 1175 および 1036 の両菌株を用いた。

培養基、培養方法、色素の同定： 前報¹⁻³⁾と同様である。なお、ペーパークロマトグラム上の色素の同定には、紫外線下の蛍光をも併用した。

紫外線変異株の作出： 分生胞子の蒸留水懸濁液をあらかじめ東洋ろ紙 No. 1 を通過させた後、適当の胞子数 ($1 \sim 3 \times 10^4/\text{ml}$) にうすめて数個のシャーレ (径 6 cm) に 10 ml ずつ分注、無菌箱内でふたを除き、30 cm の距離から紫外線ランプ (15 W) で一定時間ずつ照射する (照射中静かにゆり動かす)。次いで、各シャーレから 0.5 ml あてとって完全培地へ接種、発生した集落のうちから、親株と異なる色調や形態を示す菌株を常法によって単離

した。

実験および結果

I. 菌株 NRRL 1175 についての実験

予備実験： 紫外線の有効な照射時間を知るために、胞子の懸濁液 ($15,370/\text{ml}$) について胞子の生存率を調べた [Fig. 1(a)]. この結果から、紫外線照射は 10 分以内にやめるべきことがわかった。

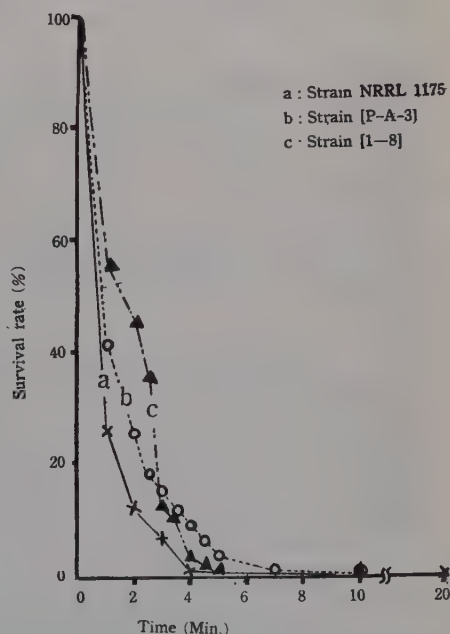


Fig. 1. Survival rate of UV-irradiated spores.

* Botanical Institute, Faculty of Science, Tokyo University of Education, Tokyo, Japan.
東京教育大学理学部植物学教室

Table 1. Defective strains obtained from the strain NRRL 1175 by UV-irradiation.

Strains	Medium	Mycelial pigments*						
		Chry	Emo	Eryth	Flav	Sky	Oxysky	Skol
[B]	Complete	+	+	—	+	+	+	+
[P-21-2]	"	+	—	+	+	+	+	+
[P-A-3]	"	+	+	—	—	+	+	+
[P-J-5]	"	—	—	—	—	+	+	+
[S-k-1]	"	+	—	+	—	+	+	+
[56]	"	+	+	—	—	+	+	+

* Chry, chrysophanol; Emo, emodin; eryth, erythroskyrin; Flav, flavoskyrin; Sky, skyrin; Oxysky, oxyskyrin; Skol, skyrinol.

このようにして分離した菌株は Table 1 のとおりである。

これらの菌株は、完全培地上 3 ~ 4 代の継代培養を重ねても、生産する色素成分には変化がみられなかった。

[P-A-3] 株の胞子への紫外線照射: 予備実験か

ら得られた [P-A-3] 株の胞子に、さらに紫外線を照射 (1, 2, 2.5, 3, 3.5, 4, 4.5, 5, 7, 9 分, おのおの 26,000/ml の胞子懸濁液) し、一見異常の色調を示す 35 株を分離した。生存率は Fig. 1(b) に示した。なお、同一条件下で別に 37 株を分離した。

Table 2-a. Defective strains obtained from the strain [P-A-3] by UV-irradiation.

Strains	Medium	Pigments*							
		Chry	Emo	Eryth	Flav	Sky	Oxysky	Skol	
[A3-1] and other 8 strains	Complete	+	+	—	—	+	+	+	1st generation
[A3-4] and other 4 strains	"	+	+	—	+	+	+	+	1st "
[A3-19] and other 10 strains	"	—	—	—	—	—	—	—	1st "
[A3-3] and other 3 strains	"	± or —	± or —	—	—	+	+	—	1st "
	"	—	—	+	—	+	+	—	2nd "
	"	—	—	+	—	+	+	—	3rd "
	"	—	—	—	—	+	+	—	4th "
	"	—	—	—	—	+	+	—	5th "
[A3-21] and other 2 strains	"	+	—	—	—	+	+	+	1st "
	"	—	—	—	—	—	—	—	2nd "
[A3-8] and [A3-24]	"	—	—	—	—	+	+	± or —	1st "
	"	—	—	—	—	+	+	—	2nd "
	"	—	—	—	—	+	+	+	3rd "
[A3-22]	"	—	—	—	—	+	+	+	1st "
	"	—	—	—	—	+	+	—	2nd "
	"	—	+	—	—	+	+	+	3rd "
	"	—	+	—	—	+	+	+	4th "
	"	—	—	—	—	+	+	+	5th "

* Abbreviations: cf. foot note of Table 1.

Table 2-b. Defective strains obtained from the strain [P-A-3] by UV-irradiation.

Strains	Medium	Pigments*							
		Chry	Emo	Eryth	Flav	Sky	Oxysky	Skol	
[a3-3] and other 3 strains	Complete	+	+	—	—	+	+	+	1st generation
[a3-5]	"	+	+	—	+	+	+	+	1st "
	"	+	+	—	+	+	+	+	2nd "
[a3-1] and other 15 strains	"	—	—	—	—	—	—	—	1st "
[a3-11]	"	—	—	—	—	+	—	—	1st "
	"	—	—	—	—	+	—	—	2nd "
[a3-8] and [a3-23]	"	± or —	—	—	—	+	—	—	1st "
	"	—	—	—	—	+	+	—	2nd "
	"	—	—	—	—	+	+	—	3rd "
[a3-19] and [a3-29]	"	± or —	—	—	—	+	+	+	1st "
	"	—	—	+	—	+	+	+	2nd "
	"	—	—	+	—	+	+	+	3rd "
	"	—	—	+	—	+	+	+	4th "
[a3-18]	"	—	—	—	—	+	+	+	1st "
	"	—	—	+	—	+	+	+	2nd "
	"	—	—	+	+	+	+	+	3rd "
	"	—	—	—	—	+	+	+	4th "
[a3-6] and other 2 strains	"	—	—	—	—	+	+	+	1st "
	"	—	+	+	—	+	+	—	2nd "
	"	—	+	+	—	+	+	+	3rd "

* Abbreviations: cf. foot note of Table 1.

これらの株について継代培養を行ない、その色素生産の消長を調べた結果を、それぞれ Table 2-a, 2-b に示した。これらの株は、最少培地では、生育がわるく、7～8週間後でも色素を生成しなかった。この際、液体培養では、生育も色素生産もきわめてわるいので、もっぱら固体培地による斜面、または平面培養を行なった。

Skyrin, oxyskyrin, skyrinol は、他の色素に比べてきわめて安定であり、この3者の消長関係をみると、skyrinol を欠く場合、skyrinol と oxyskyrin との2者を欠く場合、skyrinol, oxyskyrin, skyrin の3者を欠く場合（この時は、菌はアルビノとなる）というように、段階的な関係がみられた。したがって、この3者は、skyrin→oxyskyrin→skyrinol の順序で出現し、しかもこの生成経路が主幹をなすものと考えられる。

これに反する事実、すなわち、skyrin の生成が

ないのに他の2者が生成されるとか、oxyskyrin の生成がないのに skyrinol が生成されるというような事例は、まったくみられなかった。

他方、chrysophanol, emodin および flavoskyrin は紫外線に対してきわめて不安定であり、かつ、3者の間には、skyrin などにみられたような段階的消長関係はみられず、たがいに独立的に出現することがわかった。

また、erythroskyrin のみを欠く場合（[A3-4] 株など）のあることは、この色素がアントラキノン色素と直接の生成的関連がないことを示している。

この際とくに注目すべきことは、[P-A-3] 株は erythroskyrin, flavoskyrin を欠くにもかかわらず、これから導かれた変異株の中には、それらを生産するようになった株が見いだされたことである。

[56] 株の胞子への紫外線照射： Table 1 の [56] 株の胞子に紫外線を照射（1, 2, 2.5, 3, 3.5, 4, 4.5, 5, 7, 9 分、おのおの 14,400/ml の胞子懸

Table 3. Defective strains obtained from the strain [56] by UV-irradiation.

Strains	Medium	Pigments*							
		Chry	Emo	Eryth	Flav	Sky	Oxysky	Skol	
[56-3] and other 22 strains	Complete	+	+	+	+	+	+	+	1st generation
[56-6] and other 31 strains	"	+	+	-	+	+	+	+	1st "
[56-2] and other 5 strains	"	-	-	-	-	-	-	-	1st "
[56-1] and other 7 strains	"	-	-	-	-	+	-	-	1st "
	"	-	-	-	-	+	+	-	2nd "
	"	-	-	-	-	+	-	-	3rd "
[56-43] and other 4 strains	"	-	-	-	-	±	±	-	1st "
	"	-	+	-	-	+	+	+	2nd "
	"	-	-	-	-	+	+	+	3rd "
	"	-	+	-	-	+	+	+	4th "
	"	-	+	-	-	+	+	+	5th "
[56-46]	"	-	+	-	-	+	+	-	1st "
[56-61]	"	+	+	-	+	+	+	+	1st "
	"	+	-	+	-	+	+	+	2nd "
	"	-	-	-	+	+	+	-	3rd "
[56-67]	"	+	+	-	-	+	+	+	1st "
	"	-	-	+	-	+	+	+	2nd "
	"	-	-	-	-	+	+	+	3rd "
[56-42]	"	+	-	-	-	+	+	+	1st "
	"	+	+	-	+	+	+	+	2nd "
	"	+	+	-	+	+	+	+	3rd "

* Abbreviations: cf. foot note of Table 1.

濁液) して 80 株を分離した。分離株の完全培地上での色素成分の消長 (Table 3) については、Table 2 と同様の傾向がみられた。

考 察 1

[P-A-3] 株と [56] 株とは、ともに erythro-skyrin, flavoskyrin を生産しないが、それらの紫外線による変異株の中には、flavoskyrin を生産するようになった株が約 40% (Table 2)、全色素成分をもつようになった株が 28% もみられた。本菌株は単核の分生孢子による無性生殖を行なうので、もし色素形成能の欠如が遺伝子突然変異によるものとすれば、以後の世代における回復の頻度はきわめて小さいはずである。したがって、[P-A-3] 株と [56] 株とにおける erythro-skyrin, flavoskyrin の欠如は、紫外線照射による孢子の細胞質の代謝機構の二次的な変化に帰すべきものと考えられる。これらの変異株を紫外線で照射するとき、色素生成能の

一部または全部が回復する事実がみられたが、この機構の解明は今後の興味ある問題である。

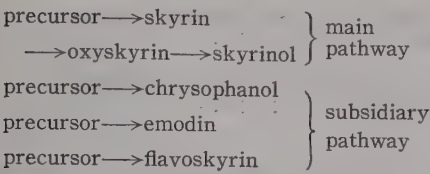
Skyrin, oxyskyrin, skyrinol の 3 者は、紫外線に対してきわめて安定であり、また、継代培養によって 3 者の段階的な消長関係がみとめられた。これは、skyrin→oxyskyrin→skyrinol の経路が主幹であるという柴田の説)を支持するものである。

一方, chrysophanol, emodin および flavoskyrin の関係については、紫外線を用いた今回の実験結果からすると、3 者がそれぞれ独立的に生産されることが明らかになった。

要約すれば、本菌株におけるアントラキノン色素群の生成的連関は、Fig. 2 のような模式によって示すことができる。

なお、すでに erythro-skyrin はアントラキノン色素とはその生成において直接的の関係のないことを述べたが¹⁻³⁾、今回の結果もまったく同様のことを示している。

Fig. 2. Hypothetical biosynthetic scheme of pigment formation in the strain NRRL 1175.



II. 菌株 NRRL 1036 についての実験

NRRL 1036 株の胞子への紫外線照射: 完全斜面培養 10~15 日の胞子に紫外線照射 (1, 2, 2.5, 3.5, 4, 5, 7, 10 分, おのおの 11,520/ml の胞子懸濁液) して 74 株を分離し, それぞれ完全, 最少両培地上で培養し, 生産する色素の消長を比較した結果を Table 4 に示した.

Skyrin, oxyskyrin は安定, islandicin, irido-skyrin は rubroskyrin, luteoskyrin よりも安定

Table 4. Defective strains obtained from the strain NRRL 1036 by UV-irradiation.

Strains	Medium*	Pigments**									
		Isl	Irid	Cat	Ery	Lut	Sky	Rub	Oxysky	Spot-j	
[1-8]	C. M.	+	+	+	+	+	+	+	+	s ***	1st generation
	M. M.	-	-	+	+	+	+	-	+	+	
	C. M.	+	+	?	+	+	+	+	+	+	2nd "
	M. M.	-	-	?	+	+	+	-	+	s	
[1-9] and [3-5a]	C. M.	+	+	?	+	+	+	+	+	s	1st "
	M. M.	-	-	+	+	-	+	-	+	s	
	C. M.	+	+	?	+	+	+	+	+	s	2nd "
	M. M.	-	-	?	+	-	+	-	+	s	
	C. M.	+	+	?	+	+	+	+	+	s	3rd "
	M. M.	-	-	+	+	-	+	-	+	+	
[1-10A] and other 3 strains	C. M.	+	+	-	+	+	+	+	+	+	1st "
	M. M.	+	+	-	+	-	+	+	+	s	
	C. M.	+	+	+	+	+	+	+	+	+	2nd "
	M. M.	+	+	+	+	+	+	+	+	+	
[3-19]	C. M.	+	+	+	?	-	+	-	+	+	1st "
	M. M.				no pigmentation occurs						
	C. M.	+	+	+	-	-	+	-	+	+	2nd "
	M. M.				no pigmentation occurs						
[3.5-13]	C. M.	+	+	+	+	+	+	+	+	+	1st "
	M. M.				no germination occurs						
	C. M.	+	+	+	+	+	+	+	+	+	2nd "
	M. M.				no germination occurs						
[3.5-14]	C. M.	-	-	-	+	-	+	-	+	s	1st "
	M. M.	-	-	-	+	-	+	-	+	s	
	C. M.	-	-	-	+	-	+	-	+	s	2nd "
	M. M.	-	-	-	+	-	+	-	+	s	
[3-16]	C. M.	-	-	-	+	-	+	-	+	s	1st "
	M. M.	-	-	-	+	-	+	-	+	s	
	C. M.	+	+	-	+	-	+	-	+	s	2nd "
	M. M.	-	-	-	+	+	+	-	+	s	
[2-7]	C. M.	+	+	?	+	-	+	+	+	s	1st "
	M. M.	+	+	?	?	+	+	-	+	+	
	C. M.	+	+	+	-	-	+	-	+	+	2nd "
	M. M.	+	+	+	-	+	+	+	+	+	

* C.M., complete medium; M.M., minimal medium.

** Isl, islandicin; Irid, iridoskyrin; Cat, catenarin; Ery, erythroskyrin; Lut, luteoskyrin; Sky, skyrin; Rub, rubroskyrin; Oxysky, oxyskyrin.

*** s, skyrinol-like pigment appears when spot-j disappears,

Table 5. Comparison in Rf-value between "yellowish brown spot" and skyrinol (Tôyô No. 53 filter paper, 13°).

	Rf-value
"Yellowish brown spot"	0.03 (Solvent: upper layer of the solvent mixture, 0.04 acetone/benzine/water (5 : 5 : 3.5, v/v)
Skyrinol (from the strain NRRL 1175)	0.03 (Solvent: upper layer of the solvent mixture, 0.04 acetone/benzine/water (5 : 5 : 3.5, v/v)

であるが, catenarin はきわめて不安定で, spot-j も消長がいちじるしい。ろ紙クロマトグラフによる調査では, spot-j の消失したあとに skyrinol に酷似する yellowish brown spot が現われる。この新色素はきわめて微量なため詳細は明らかでないが, 再抽出法によって試験すると, Table 5 に示したように, Rf 値は skyrinol のそれとまったく一致する。Rubroskyrin と luteoskyrin との相互関係についてみると, 両者の間には明らかにせりあいの関係がある。すなわち, [1-8] 株では, 最少培地上で luteoskyrin の生産はみられるが, rubroskyrin の生産はみられない。

また, [3-16] 株でも 2 代目の最少培地上で luteoskyrin の生産はみられるが, rubroskyrin の生産はみられなかった。

これとは逆に, [1-10A] はか 3 株の 1 代目では最少培地上で rubroskyrin の生産はみられるが, luteoskyrin の生産はみられない。なお, [2-7] 株の 1 代目では, 完全培地で rubroskyrin の生産がみられ, luteoskyrin の生産はみられないが, 最少培地では rubroskyrin の生産がみられず, luteoskyrin の生産がみられた。

[3-16] 株の胞子への紫外線照射: Table 4 の [3-16] 株の胞子に紫外線照射 (1, 2, 3, 3.5, 4, 4.5, 6, 8, 10 分, おおの 10,800/ml の胞子懸濁液) して 66 株を分離し, 色素成分を調べたところ, 全株とも erythroskyrin, skyrin, oxyskyrin, skyrinol-like pigment を生産するだけであった (Table 6)。

この 66 株から任意に 12 株をえらんで 2 代目の

Table 6. Pigment production in defective strains derived from the strain [3-16].

Strains	Medium*	Pigments**								
		Isl	Irid	Cat	Ery	Lut	Sky	Rub	Oxysky	Spot-j
All (66) strains obtained	C. M.	—	—	—	+	—	+	—	+	s ***
	M. M.	—	—	—	+	—	+	—	+	s

*, **, *** Cf. foot note of Table 4.

Table 7. Pigment production in the second generation of 12 strains (taken out of 66 strains shown in Table 6).

Number of strains	Medium*	Pigments**								
		Isl	Irid	Cat	Ery	Lut	Sky	Rub	Oxysky	Spot-j
10	C. M.	—	—	—	+	—	+	—	+	s ***
	M. M.	—	—	—	+	—	+	—	+	s
1	C. M.	—	—	+	+	—	+	—	+	s
	M. M.	—	—	+	+	—	+	—	+	s
1	C. M.	+	—	—	+	—	+	—	+	s
	M. M.	—	—	—	+	—	+	—	+	s

*, **, *** Cf. foot note of Table 4.

Table 8. Pigment production in defective strains induced from the strain [1-8].

Strains	Medium*	Pigments**								
		Isl	Irid	Cat	Fry	Lut	Sky	Rub	Oxysky	Spot-j
[1-1] and other 40 strains	C. M.	+	+	—	+	+	+	+	+	s ***
[3-1] and other 38 strains	"	+	+	+	+	+	+	+	+	s
[2.5-17] and other 2 strains	"	+	—	+	+	+	+	+	+	s
[3-4]	"	—	+	—	+	?	+	+	+	s
[2.5-13]	"	+	+	—	+	—	+	—	+	s
[3.5-15]	"	+	+	+	—	—	+	—	+	s
[2-20]	"	+	+	—	—	+	+	+	+	s
[2-17] and [2-19]	"	+	+	—	+	—	+	+	+	s
[3-19]	"	+	+	—	+	+	+	—	+	s
[3-7] and other 11 strains	"	+	+	+	+	+	+	+	+	+

*, **, *** Cf. foot note of Table 4.

色素成分を調べたところ、10 株は 1 代目と同じであり、1 株は catenarin の生産がみられるようになり、他の 1 株は完全培地上で islandicin の生産がみられるようになった (Table 7)。

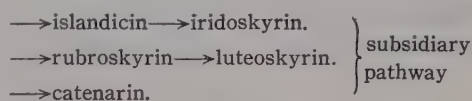
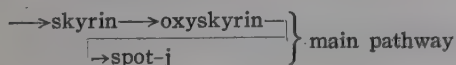
[1-8] 株の胞子への紫外線照射: Table 4 の [1-8] 株の胞子に紫外線を照射 (2, 2.5, 3, 3.5, 4, 4.5, 5, 6, 8, 10 分, おのおの 14,400/ml の胞子懸濁液) し、102 株を分離した。完全培地上におけるこれら菌株の色素生産の消長は Table 8 に、また生存率は Fig. 1(c) に示した。

この場合も、rubroskyrin (+), luteoskyrin (—) の株 ([2-17] 株など) や、rubroskyrin (—), luteoskyrin (+) の株 ([3-19] 株) があつた。

Islandicin と iridoskyrin との関係については、islandicin (+), iridoskyrin (—) の株 ([2.5-17] 株など) や、islandicin (—), iridoskyrin (+) の株 ([3-4] 株) がみられた。すなわち、これら両色素の生成にもせりあいの関係がみられる。

考 察 2

前報³⁾では、本菌株の色素の生成的連関について次のような経路を提案した。



紫外線を用いての本実験からも (rubroskyrin, luteoskyrin) と (islandicin, iridoskyrin) との両群はそれぞれ独立的に生産されることがみられたが、さらに、rubroskyrin (—) のときでも luteoskyrin (+) の株 ([1-8] 株, Table 4), また、luteoskyrin (—) のときでも rubroskyrin (+) の株 ([1-10A] 株, Table 4) が得られているので、rubroskyrin と luteoskyrin とは共通の先駆物質からたがいに並行的に生成されるものと考えられる。おそらく、菌の発育条件によって、両者が並行して生成されたり、または一方の消失が起こるものと思われる。Islandicin と iridoskyrin との場合もまた共通の先駆物質から並行的に生成されるものと考えられる。

最近、Gatenbeck⁵⁾ は、ラベルした酢酸を用いた実験から、(islandicin—iridoskyrin) と (rubroskyrin—luteoskyrin) との 2 群は、それぞれ先駆物質を異にすること、また、islandicin ⇌ iridoskyrin の経路は存在しないと述べており、これは著者らの結果とも矛盾しない。

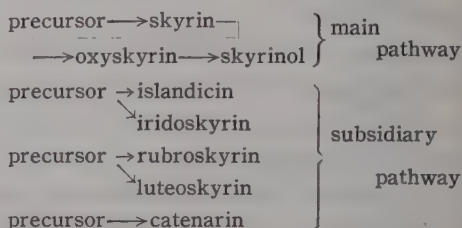
ろ紙クロマトグラム上で、spot-j の消失したあと

に現われる spot は, NRRL 1175 株にみられる skyrinol とよく似ている。この色素が spot-j に由来するものか, あるいは, もともと spot-j によっておおわれた微量の skyrinol が spot-j の消失によって現われてきたものか, いずれとも断定できないが, NRRL 1175 株に skyrin, oxyskyrin (2-hydroxymethylskyrin), skyrinol (2,2'-dihydroxymethylskyrin) が存在し, NRRL 1036 株にも skyrin, oxyskyrin が生成されることをあわせ考えると, NRRL 1036 株にもまた微量の skyrinol があり spot-j によっておおわれている可能性が考えられる。Table 5 に示された結果は, これを裏書きしているように見える。したがって, spot-j は skyrin \rightarrow skyrinol に至る主経路から, はずれた別の経路に属するものであろう。

以上の考察に基づいて, 著者らは本菌株における色素相互間の生成的連関について, 次のような模式を提出する。これによって, skyrin, oxyskyrin が発育の早期に出現し, しかもきわめて安定なこと, islandicin と iridoskyrin, rubroskyrin と luteoskyrin がそれぞれたがいに緊密な競合関係を示す

こと, また, islandicin-iridoskyrin 群が rubroskyrin-luteoskyrin 群よりも安定であり, かつ, 両群はそれぞれ独立的に消長することなどが, この図式によってよく理解されるであろう。

Fig. 3. Hypothetical biosynthetic scheme of pigment formation in the strain NRRL 1036.



総 括

Penicillium islandicum Sopp. は色素成分によって NRRL 1175, 1036, LSHTM BB 233 の 3 chemical strains に分けられている。LSHTM BB 233 株は erythroskyrin を生産するという点で他の 2 菌株と関連しているが, erythroskyrin はアン

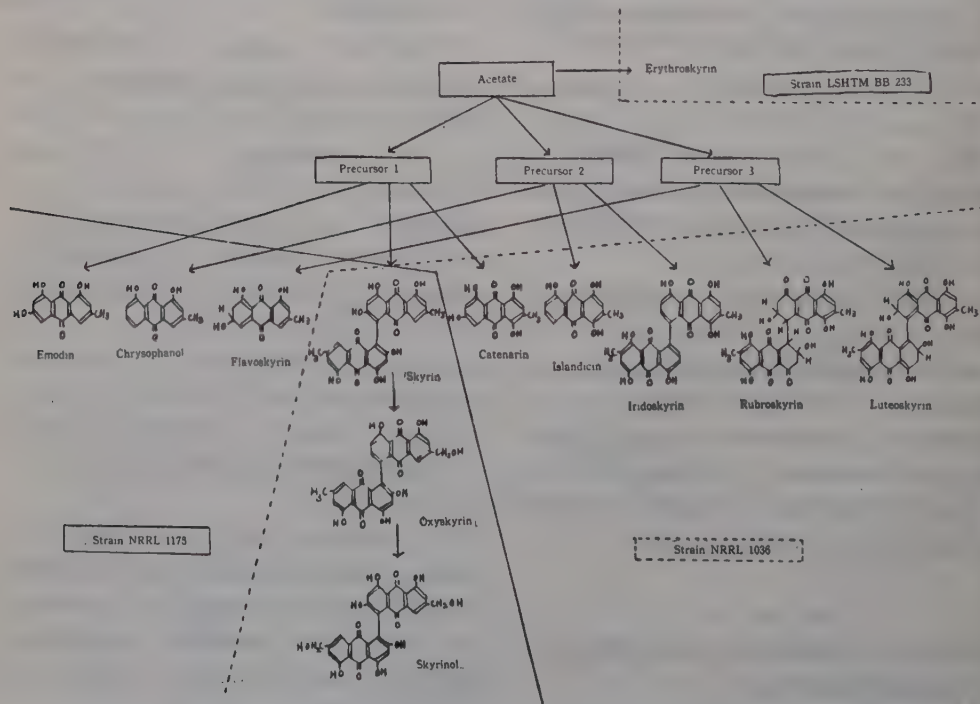


Fig. 4. Biosynthetic relationships of pigment formation in three chemical strains of *P. islandicum* Sopp.

トラキノン色素とは生成的には関係のないことが示されており、このことはまた、紫外線照射の実験からも明らかにされた。

ここで、アントラキノン系色素の生成的連関について、NRRL 1175 および 1036 両菌株を用いて行なった研究結果を総合して考察してみたい。

両菌株の生産する色素の化学構造を比較対照すると、次のような注目すべき諸点が指摘される。

1) Catenarin は emodin の α -hydroxyl 置換体であり、skyrin は emodin の dimer である。

2) Islandicin は chrysophanol の α -hydroxyl 置換体であり、iridoskyrin は islandicin の dimer である。

3) Luteoskyrin は flavoskyrin の α -hydroxyl 置換体の dimer とみなされ、また、rubroskyrin の脱水によっても容易に形成される。

これらの構造上の知見と、考察 1, 2 で示した生成経路とを結びつけると、*Penicillium islandicum* Sopp. における色素の生成的関連は Fig. 4 のように表わされる。

したがって、色素の生成に關して、NRRL 1175 株と NRRL 1036 株とのいちじるしい相違点は、前者に欠けている酸化系が後者では備わっていることである。また、きわめて興味のあることは、もっとも強力であり安定である skyrin \rightarrow oxyskyrin \rightarrow skyrinol の経路を両菌株が共有していることである。

この研究に際して、終始ご指導を賜った東京教育大学理学部の林孝三教授と有益なご助言を賜った東京大学薬学部の柴田承二教授に厚く謝意を表する。

References

- 1) Hayashi, K., Kikuchi, M., and Okamoto, Y., Bot. Mag. Tokyo **72**: 220 (1959).
- 2) Kikuchi, M., Okamoto, Y., and Hayashi, K., *ibid.* **73**: 195 (1960).
- 3) Kikuchi, M., *ibid.* **74**: 42 (1961).
- 4) Shibata, S., Kagaku (in Japanese) **26**: 391 (1956).
- 5) Gatenbeck, S., Acta Chem. Scand. **14**: 102 (1960).

Summary

On the basis of the experiments on pigment formation in several strains obtained from *Penicillium islandicum* Sopp. by UV-irradiation, biosynthetic interrelationships of the pigment components may be summarized as follows:

1) The strains obtained by irradiation are not mutants caused by gene mutation, but defective strains due to cytoplasmic change in irradiated spores.

2) In the strain NRRL 1175, the pathway leading to skyrin, oxyskyrin and skyrinol is found to be quite stable against UV-irradiation, and the loss or recovery of individual pigments has been achieved step by step during successive culture. Therefore, these 3 pigments are involved in the main pathway which proceeds according to the following fashion: skyrin \rightarrow oxyskyrin \rightarrow skyrinol. However, chrysophanol, emodin and flavoskyrin are unstable, and the appearance or disappearance of these pigments takes place independently of each other.

The biosynthetic route of the pigments in the strain NRRL 1175 is shown in Fig. 2 in the text.

3) In some defective strains obtained from the strain NRRL 1036, a faint new pigment spot comes into appearance in place of spot-j on the chromatogram only after disappearance of the latter pigment. This new pigment was identified as skyrinol by paper chromatography (Table 5). Thus, it is likely that skyrinol is also present in the strain NRRL 1036 probably being masked by preponderant spot-j on the chromatogram. Therefore, the route, skyrin \rightarrow oxyskyrin \rightarrow skyrinol, is the main pathway also in this strain.

Islandicin and iridoskyrin are formed from a common precursor in a competitive manner. The interrelationship between rubroskyrin and luteoskyrin is quite similar to that between islandicin and iridoskyrin.

In view of these experimental findings, a scheme of pigment synthesis in the strain NRRL 1036 is illustrated in Fig. 3 in the text.

4) Erythroskyrin stands outside the biosynthetic route of anthraquinones.

5) The most plausible feature of the biosynthesis of a group of anthraquinones in the two chemical strains of *Penicillium islandicum* Sopp. is mapped out in Fig. 4 in the text.

本 会 記 事

支部通信

北海道支部

支部大会 (昭和 36 年 7 月 8・9 日 帯広畜産大学において)

桑山弥寿男 (北海道学芸大岩見沢分校)・庄貞行 (北大理植)・宇佐美正一郎 (北大理植)：ミズバショウ花穂の呼吸にみられる青酸促進について、大野林二郎・武久 慎 (北大理植)：水浸・温度処理の組み合わせによる *Trillium* の meiotic division の異常について、松本光治 (小樽潮陵高)：羊蹄山登山路植物景観、松浦 一・武久 慎 (北大理植)： *Petunia* の花色の遺伝 I. 花色発現に関する genetic constitution が同一と考えられる S1-population における個体間の paper chromatographic な差について、松浦 一・武久 慎 (北大理植)： *Trillium* の meiotic metaphase I の染色体構造におよぼす EDTA の効果、寺岡 宏 (北星短大)：発芽コムギ胚におけるサッカラーゼ活性と生長との関係、沢田義康 (北海道学芸大旭川分校)：雌ずいおよび雄ずいにふくまれるオーキシン含量について、佐々木勝治 (北海道学芸大旭川分校)：ハクサイの結球現象とオーキシンおよびジベレリン含量について、後藤寛治 (北海道農業試験場十勝支場)：ダイズ奥原 1 号の表現型変更と環境条件、[特別講演] 松浦 一 (北大理植)：現代における生物学の一つの問題点——ソビエト・中国における生物学を中心として、池田好郎 (十勝農高)：知床学術調査報告

9 月例会 (9 月 6 日 北大農学部において)

Wildman (カリフォルニア大)：植物ミトコンドリアの形成過程 (9 月 27 日 北大農学部において)、館脇 操：ハワイの花、E. Hultén (スウェーデン国立博物館)：極北アラスカについて

東北支部

支部大会 (昭和 36 年 8 月 26・27 日 新庄南高校において)

渡辺 仁 (東北大理)：オジギソウの刺激物質について、中沢 潤 (弘前大文理)：高温処理による花粉内異常核分裂、柴岡孝雄 (東北大理)：オジギソウ興奮性細胞の活動電位、小田健二 (東北大理)：シャジクモの活動電位波形の解析、田中 清 (福島大学芸)：アカマツ花粉にふくまれる生長抑制物質のカブ種子の発芽におよぼす影響、中沢信午 (山形大文理)：形態形成の場ではアインシュタインのブラウン運動方程式がかならずしも成立しないこと、遠田宏 (東北大理)：セリバオウレンの性の表現について、林 義昭 (東北大理)：シキミの胚のう形成および胚発生について、武内康義 (東北大理)：青森県湯の島の植物、吉岡邦二 (東北大理)：東北地方におけるブナ分布の下限、樋口利雄 (福島県松川産高)：福島県田村郡における石灰岩地帯のセン類、飯泉 茂 (東北大理)：野火跡地における灌木類の再生について、櫻村利道 (福島大学芸)：冬期におけるアカマツ林植物の日補償点、山本 弘 (岩手県宮古高)：北上山地東側におけるウリハダカエデの分布

関東支部

9 月例会 (9 月 30 日 東大理学部において)

百瀬静男 (文部省)：リュウビンタイの配偶体について——シダ類の配偶体の形態分類、服部静夫 (東大理植)：IUBS 第 14 回総会に出席して

近畿支部

7 月例会 (7 月 1 日 奈良女子大において)

衣川堅二郎 (京大農応用植物)：アカマツ幼植物の主軸伸長と菌根形成におよぼす日長・温度・光量の影響、小川幸持 (京大農応用植物)：ダイコンの開花に伴う生長素およびジベレリン様物質の消長、菅沼孝之 (奈良女子大理植)：本州におけるナガバノモウセンゴケとサジバノモウセンゴケの新産地について

公開講演 (日本生態学会近畿支部と共催。9 月 13 日 関西日仏学館ホールにおいて)

H. Gaussen (ツールーズ理科大)：フランスの植物生態分布地図

Ecological Studies of *Sasa* Communities

IV. Dry Matter Production and Distribution of Products among Various Organs in *Sasa kurilensis* Community*

by Yasuyuki OSHIMA**

Received March 22, 1961

Production and reproduction of dry matter, determining the productive structure of the next production term, are most fundamental and important for growth analysis of a plant community and furthermore for analysis of social relationships, such as competition, succession, and stability, in plant community, related to environmental factors. Therefore, dynamic aspect of development of plant community should be elucidated on the basis of matter production and reproduction.

On this line, valuable studies¹⁻¹⁴) were recently made, and concerning matter reproduction some schemata were submitted to clarify some important ecological problems^{9,15}).

The author has already made analytical studies on *Sasa* communities in central and northern Japan, and clarified their specific features of physiological functions and productive structures¹⁶⁻¹⁸).

In the present work, the estimation of dry matter production and the distribution of produced matter among organs of *Sasa kurilensis* community, especially of Mt. Waisuhoron in southern Hokkaido, will be performed with special reference to their characteristics.

1. Dry matter production

Direct field measurement of the total amount of photosynthesis or of gross production (P_g) of large plant community is difficult. Recently, the relationship among leaf area index F , vertical light distribution and total photosynthesis in a closed plant community was studied^{14,19,20}), and Saeki's formula²⁰) proposed for P_g calculation is as follows:

$$\text{Daily } P_g = \frac{b}{Ka} \ln \frac{(1-m) + KaI_0}{(1-m) + KaI_0 \exp(-KF)}$$

where K is extinction coefficient, m , light transmissibility of a leaf, I_0 , the light intensity at the top of the community, and a and b are the constants which characterize the shape of the daily photosynthesis-relative light intensity curve. With this equation, the author calculated the P_g of the *Sasa kurilensis* community in a fine and a cloudy day from May 23 to November 7, using K , F and m shown in a previous paper¹⁷), and a and b obtained from the curve in Fig. 3 of another paper¹⁸). The light intensity of the cloudy day was assumed here to be 1/5 of that of the fine day.

Monthly gross production in those periods (Fig. 1) was calculated from daily gross production with correction using the monthly duration of bright sunshine observed at Kutchan Meteorological Station situated about 12 km. far from the stand in question. Then the mean daily gross production was obtained (Fig. 1). The highest daily gross production during the growing period, 36 g./m.²/day, was obtained in May, and the

* A part of expense of this study was supported by a Grant-in-Aid from the Research Fund of the Ministry of Education.

** Department of Biology, Faculty of Science, Tokyo Metropolitan University, Setagaya, Tokyo, Japan.

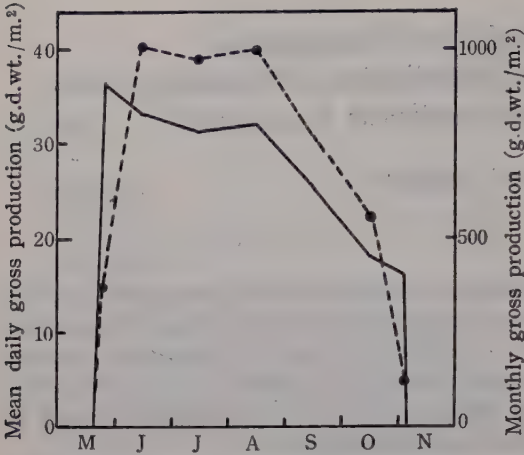


Fig. 1. Seasonal changes in the monthly (broken line) and mean daily (solid line) gross production of a *Sasa kurilensis* community at Mt. Waisuhorun, southern Hokkaido, from May 23 to November 7 of 1959.

lowest, 16 g./m.²/day, in November. The sum total of the monthly gross production gives the annual gross production of about 4.76 kg./m.², the mean daily production being about 28 g./m.² during the growing period of about 170 days, from May 23 to November 7.

The daily maximum, minimum and mean temperatures at Mt. Waisuhorun were estimated from the data measured at the said station with a mean lapse rate of 0.55°/100 m. A good coincidence between the estimated mean temperature and that observed directly in the *Sasa* community was proved as seen in Table 1. The mean daytime temperature important for photosynthetic activity was, without direct measurement, assumed to be

nearly the same as the mean value of the daily mean and the daily maximum temperature, and it was calculated for the every period of 10 days during the growing season (Fig. 2). These values were distributed in the range of 14°~21° except for the depressed values in October and November. The temperature coefficient of photosynthesis is relatively small and the optimal temperature of photosynthesis of subalpine plants is found at about 18° or less^{11,21,22}). These facts may give an expectation that the *Pg* calculated at 20° without correction to temperature fluctuation does not much differ from the real *Pg* brought forth in the nature.

The temperature coefficient of respiration is so large that the respiration at 20° should be corrected according to the temperature at the station¹⁸). For this correction, the mean temperatures for every period of 10 days as calculated above, and the soil temperatures measured in every season at 20 cm. depth where the most of subterranean parts were distributed, were used (Fig. 2).

In winter season the culms are bent down to keep their height at about 60-70 cm. above the ground under the snow cover of 2 m. or more (see Fig. 3 in a previous paper¹⁷). The temperature in the accumulated snow was -1° at 30 cm. depth and increased slightly with snow depth (see Fig. 2 in the same paper¹⁷). So the tem-

Table 1. Comparison between the temperatures calculated from the data measured at Kutchan Meteorological Station and those observed at 1 m. height in a *Sasa kurilensis* community. At Waisuhorun, southern Hokkaido.

Date	Daily maximum		Daily minimum		Daily mean	
	Calculated	Observed	Calculated	Observed	Calculated	Observed
June 2, 1959	11.9	10.8	9.6	9.9	10.1	10.2
Aug. 9	21.8	19.8	10.9	13.2	16.3	16.5
Oct. 13	15.8	12.5	3.9	4.6	8.0	7.9

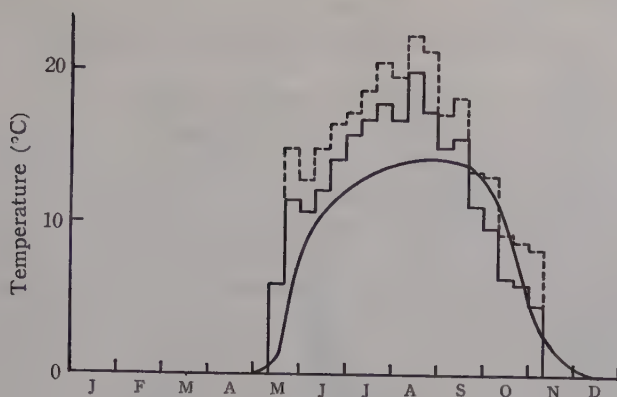


Fig. 2. Seasonal changes of mean air temperatures (averaged for 10 days: solid line), estimated mean daytime temperatures (broken line) and soil temperatures at the depth of 20 cm. (smooth curve) in the *Sasa kurilensis* community. These temperatures are calculated from the data of Kutchan Meteorological Station.

perature factor for *Sasa kurilensis* under snow cover was assumed to be 0° .

The mean respiration loss in each organ of each age per lm^2 stand in every period of 10 days at 20° was calculated by the same method as described in a previous paper¹²⁾ (cf. Figs. 4 and 5 in a previous paper¹⁸⁾), using the data of seasonal changes in dry weight (Fig. 3 in the present paper, and Figs. 1 and 4 in a previous paper¹⁷⁾). The calculated values were corrected with Q_{10} of respiration¹⁸⁾, to obtain the daily respiration of each organ under field conditions (Fig. 4). The respiration of the *Sasa kurilensis* community had two maxima of $18.6 \text{ g./m}^2/\text{day}$ in mid-July and mid-August, caused by the high respiration of each organ¹⁸⁾ and the maximum of mean air temperature, respectively. Respiration in winter was $4.0 \text{ g./m}^2/\text{day}$ or only 22% of the summer maxima. Annual respiration loss of the whole community was 3.03 kg./m^2 , which corresponded to about 65% of the annual gross production (Table 2).

The annual net production which was determined as the difference between the annual gross production and respiration loss was 1.73 kg./m^2 . This value coincided well with the annual net production of 1.6 kg./m^2 , which was directly determined on the basis of the annual increment in dry matter (Table 6 of a previous paper¹⁷⁾).

The character of these values can be distinguished by comparing with those obtained in plant

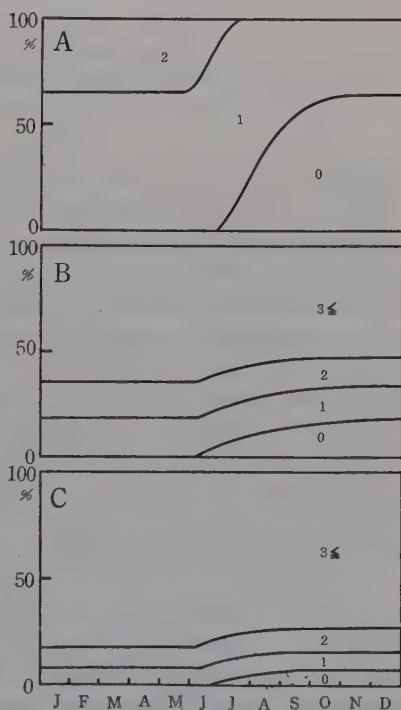


Fig. 3. Seasonal trends of the dry weight percentage of leaves (A), branches (B) and main culms (C) of each age. Numerals in the figure indicate ages.

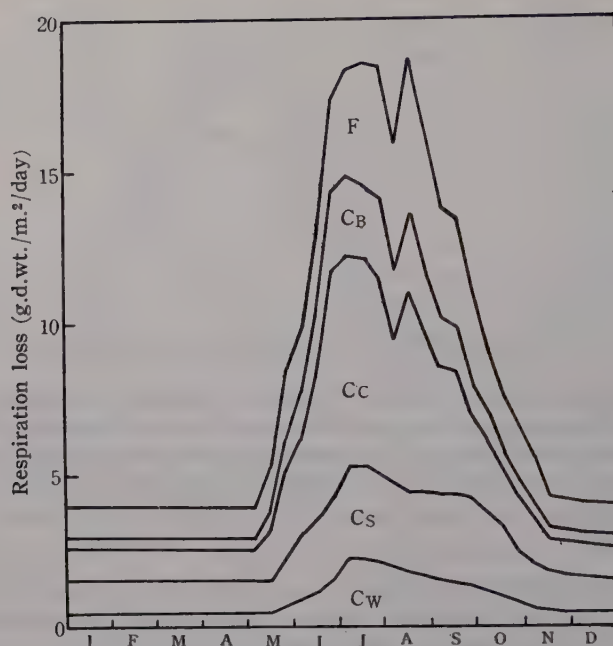


Fig. 4. Seasonal changes of daily respiration loss of leaves (F), branches (C_B), culms (C_C), rhizomes (C_S), and roots (C_W) in a *Sasa kurilensis* community. Respiration loss is shown in mean values for ten days.

communities in cool- and warm-temperate zones. In cool-temperate zone, the amounts of annual gross production, net production and respiration loss per $m.^2$ were respectively 2.85 (including shedding part of 0.45 kg.), 1.55 and 0.85 kg. in an *Acconitum* community at Mt. Hakkōda in northern Honshū⁹); 3.15, 1.80 and 1.35 kg. in a 40-year-old *Picea* forest in Denmark⁵) (computed by the present author by an assumption that leaf respiration/total respiration = 0.36¹¹), and 1.0, 0.5 and 0.5 kg. in *Solanum nodiflorum*²). It is very characteristic that the *S. kurilensis* community is extremely higher in the annual gross production and respiration loss than other plant communities which develop under similar temperature conditions. For such high gross production the high photosynthetic activity of leaves during the growing season^{17,18}), and relatively long production period of 6 months with the community structure in active state¹⁷) are responsible. The large amount of nonphotosynthetic system keeps its respiration at certain value even after three years or later¹⁸).

The larger values of gross production and respiration loss were naturally seen in the warm-temperate evergreen broad-leaved forest at the Ōsumi Peninsula, the southern-most part of Kyūshū, the former was 7.3–7.5 kg./ $m.^2$ /year¹³). These high values in Ōsumi may be brought forth by high temperature (annual mean is ca. 17°), long production period, and large standing crop of photosynthetic and non-photosynthetic systems.

Among various values of daily gross production ever reported, one of the highest was 50 g./ $m.^2$ /day of the artificial community of *Helianthus tuberosus*¹²). In the *Sasa kurilensis* community the maximum daily gross production was 36 g./ $m.^2$ and in

Aconitum in Hakkōda 35 g./m.²⁹). These seem to be the highest values observed in natural plant communities in the montane region.

2. Distribution of products among various organs

The standing crop of a plant community is determined by, besides the amount of gross production, the following items; rate of distribution of products to each organ, its respiration loss and longevity. In order to make clear the interrelations between these items, Table 2 was prepared. In these relations, provided constant gross production, the amounts of annual respiration loss of leaves (R_f) and that of total plant (R_i) are principally important in determining the amount of annual surplus production (P_s) and net production (P_n); because $P_s = P_g - R_f$, and $P_n = P_g - R_i$. In the *Sasa* community the ratio R_i/P_g was about 65%. This is a considerably large value in comparison with those values measured in herbs, e.g. 30% in *Aconitum*⁹), 50% in *Solanum*²), ca. 50% in *Helianthus*¹²), and in trees, e.g. 42% in a 46-year-old *Fagus*⁵), 50% in a 20-year-old *Abies* forest¹¹).

Table 2. Annual net production, gross production, respiration loss of the *Sasa kurilensis* community at Waisuhorun, southern Hokkaido. The numbers in parentheses indicate the relative values to gross production of the community (4.63 kg./m.²). As to annual net production, see Tab. 6 in a previous paper¹⁷.

	Whole	Leaves	Culms	Branches	Rhizomes	Roots
Annual net production (P_n) in kg./m. ²	1.6 (0.35)	0.1 (0.07)	0.54 (0.12)	0.32 (0.07)	0.32 (0.07)	0.11 (0.02)
	100%	19%	34%	20%	20%	7%
Annual respiration loss (R_i) in kg./m. ²	3.03 (0.65)	0.735 (0.16)	0.955 (0.20)	0.36 (0.08)	0.655 (0.14)	0.325 (0.07)
	100%	24%	31%	12%	22%	11%
Annual gross production ($P_n + R_i$) in kg./m. ²	4.63 (1.00)	1.045 (0.23)	1.495 (0.32)	0.68 (0.15)	0.975 (0.21)	0.435 (0.09)

The ratio R_f/P_g is 16% in *Sasa kurilensis* and 22% in *Aconitum*, 20% in *Fagus*, and 18% in *Abies* which has long-lived needles. The small value in the *Sasa* is probably caused by the mean leaf-respiration rate that is lowered by the low respiration in two-year-old leaves. The high value of the ratio R_i/P_g , in spite of the advantageous character for high P_s caused by small R_f/P_g , of this community is due mainly to the large respiration loss of non-photosynthetic system (C_r), which is derived from the large amount of C and the specificity in functional property¹⁸), and it must be rather disadvantageous in the economic life of *S. kurilensis*.

As discussed above and in a previous paper¹⁷), P_n of this *Sasa* community is, despite of the large amount of R_i , relatively large in comparison with P_n of other herb or of tree communities measured under similar environmental conditions. This is obviously due to large P_g .

The amounts of transformation of P_g to the photosynthetic and non-photosynthetic systems are also important for the development of both systems. Therefore, the ratios of newly formed photosynthetic (ΔF) and non-photosynthetic systems (ΔC) to P_n should be analysed. Although the P_n value of *S. kurilensis* community is considerably large, the ratio of new leaves to P_n ($\Delta F/P_n$) is 19% and rather small compared with other deciduous plant communities^{2,5,7,9,12,13}). This is owing

to the longevity of leaf lasting for two years. As pointed out by Monsi¹⁵) this specificity of *Sasa* leaves may be able to have an advantageous effect when low productivity is expected in certain circumstances.

Furthermore, the reserve substance plays an important part in reproductive process of produced matter^{9,15,17}). The transformation factor of ca. 0.5 was observed in the *Sasa* community¹⁷). The ratio of the dry matter of new organs transformed from reserve substances in rhizomes and old culms to P_n is about 35%, and the ratio of the reserve substances to P_g is about 25%; 1/4 of the total products is reserved in the storage organs till the end of spring of the next year.

The large standing crop of *Sasa kurilensis* is due to large P_n and large proportion of P_n translocated into non-photosynthetic system C , which has a long life span often of about ten years (see Table 2 of this paper and Fig. 4 of a previous paper¹⁶)).

The ratios C/F , $P_g/(F+C)$ and $P_n/(F+C)$ in *Sasa kurilensis* community were, respectively, a large value of 25 and small values of 0.4 and 0.14. In herb communities, almost all aerial parts generally wither and are lost every year, and the standing crop is rather small irrespective of the high P_n , and the C/F -ratio is small during the growing period, as compared with *S. kurilensis*. That the new culms of *S. kurilensis* sprout out directly from rhizomes suggests some similarity to perennial herbs in the mode of matter reproduction. *Sasa*, however, differs distinctly from common perennial grasses in such characters as longevity of C , large C/F -ratio, small $P_n/(F+C)$, evergreenness of leaves, tall height of plants, branching on older culms, etc. Therefore, it may be concluded that *S. kurilensis* is more resemble in life type shrub and trees than common perennial grasses and herbs, in spite of no marked thickening and elongation of *Sasa* culms older than two years. Other species of *Sasa* which bear culms of shorter longevity, e.g. *S. nipponica*,¹⁸) resemble rather common perennial herbs in the community structure with small standing crop, small height, and small C/F -ratio, and in the mode of matter reproduction.

Summary

Dry matter production and distribution of products to each organ were studied in a closed community of *Sasa kurilensis* at Mt. Waisuhoron, southern Hokkaido.

1. The daily maximum and annual gross production were calculated from photosynthetic curves, leaf area index, and light intensity 36 g./m.², and 4.76 kg. d.wt./m.², respectively. This high gross production may result from the high photosynthetic activity of leaves for two years, and from the maintenance of active productive structure throughout the 6-month growing period.

2. Daily respiration loss indicated a maximum of 18.6 g.d.wt./m.² in summer, and a minimum of 4.0 g.d.wt./m.² in winter, and the annual total reached 3.03 kg.d.wt./m.² Such large respiration loss was mainly due to the large amount and high respiration rate of the non-photosynthetic system.

3. Annual net production, the balance between annual gross production and respiration loss, was 1.73 kg./m.²; this is only slightly higher than the value 1.60 kg./m.² determined directly from the annual dry matter increment.

4. New organs transformed from reserve substances in rhizomes and older culms were in dry weight ca. 35% of annual net production, and 25% of annual gross production was stored as reserve substances.

5. The large net production and the large distribution ratio of products to non-photosynthetic system (especially culms) with long life of ca. 10 years must be responsible for the large standing crop of this *Sasa* community.

The author should like to express his sincere thanks to Prof. K. Hogetsu and Assistant Prof. Y. Kitazawa of Tokyo Metropolitan University and Prof. M. Monsi of the University of Tokyo for their valuable advice and suggestion.

References

- 1) Boysen Jensen, P., Die Stoffproduktion der Pflanzen, Jena (1932). 2) Larsen, P., *Planta* **32**: 341 (1941).
- 3) Möller, C. M., *Det forstl. Forsøgsv.*, Danmark **17**: 1 (1944). 4) Monşi, M., and Saeki, T., *Jap. J. Bot.* **14**: 22 (1953).
- 5) Möller, C. M., Müller, D. and Nielsen, J., *Det forstl. Forsøgsv.*, Danmark **21**: 253, 273, 327 (1954). 6) Monsi, M., and Oshima, Y., *Jap. J. Bot.* **15**: 60 (1955).
- 7) Iwaki, H., *ibid.* **16**: 210 (1958). 8) —, *ibid.* **17**: 120 (1959).
- 9) Midorikawa, B., *Ecol. Rev.* **15**: 83 (1959). 10) Kuroiwa, S., *Bot. Mag. Tokyo* **72**: 413 (1959).
- 11) —, *ibid.* **73**: 133, 165 (1960). 12) Hogetsu, K., Oshima, Y., Midorikawa, B., Sakamoto, M., Tezuka, Y., Mototani, I., and Kimura, M., *Jap. J. Bot.* **17**: 278 (1960).
- 13) Kimura, M., *Misc. Rep. Res. Inst. Natur. Resour.* **52-53**: 36 (1960). 14) Tezuka, Y., *Jap. J. Bot.* **17**: 371 (1961).
- 15) Monsi, M., *Bot. Mag. Tokyo* **73**: 81 (1960). 16) Oshima, Y., *ibid.* **74**: 199 (1961).
- 17) —, *ibid.* **74**: 280 (1961). 18) —, *ibid.* **74**: 349 (1961).
- 19) Davidson, J. L., and Philip, J. R., *Climatol. and Microclimatol.* UNESCO **181** (1958).
- 20) Saeki, T., *Bot. Mag. Tokyo* **73**: 55 (1960).
- 21) Tranquillini, W., *Planta* **46**: 154 (1955).
- 22) Pisek, T., and Winkler, E., *Planta* **51**: 518 (1958).

摘 要

大 島 康 行: ササ群落の生態学的研究 IV. チンマザサ群落の物質生産と生産物の各器官への分配

すでに報告した¹⁵⁻¹⁸⁾ササ群落の生産構造と生理機能の解析結果を基礎にして、北海道ワイスホルン山のよく発達したおなじチンマザサ群落の物質生産をもとめ、さらに生産された物質の各器官への分配を量的に解析した。

月平均の最高の日総生産量、生育期間を通じての平均の日総生産量、および年総生産量は、乾物あたりそれぞれ 36 g./m.^2 , 28 g./m.^2 , 4.76 kg./m.^2 に達した。この高い総生産量はおもに、約6か月の生育期間中、葉の高い光合成能力とその生産構造が一定の活動的な状態に維持されているためである。一方、1日あたりの呼吸消費量は乾物で夏季に最高の 18.6 g./m.^2 冬に最低の 4.0 g./m.^2 、年呼吸消費量は 3.03 kg./m.^2 であった。この高い値はおもに非同化器官の量が大いこと、およびその高い呼吸能¹⁸⁾のためである。年総生産量と年呼吸消費量の差から得られた年純生産量は 1.73 kg./m.^2 となり、すでに報告した¹⁷⁾各器官の年間の増分から直接に得た年純生産量 1.6 kg./m.^2 とほぼ同じである。

年総生産量のうち年呼吸消費量の割合は65%、また新しい葉の生産と、葉の呼吸に使われる割合は22%であった。また年呼吸消費量のうち葉の呼吸消費量の占める割合は24%、年純生産量に対する新生葉の割合は19%であった。年総生産量のうち25%は地下部と古い桿に貯蔵物質として貯蔵され、これら貯蔵物質のうち翌年新しい器官の形成に使われる量は年純生産量の約35%にあたる。

チンマザサ群落の示す高い現存量は、おもに高い純生産量と、純生産量の非同化器官への分配の割合の大きいこと、および非同化器官の寿命が約10年であることによっていることが明らかになった。

(東京都立大学理学部生物学教室)

Studies on Graft Hybrids of *Capsicum annuum* L. II. Variation in Fruit Shape Caused by Grafting for Three Successive Generations and the Effects in the Progeny*

by Noboru YAGISHITA**

Received June 1, 1961

In the preceding paper¹⁾ the author has reported that some conspicuous variation in fruit shape was caused by grafting, and that these characters were transmitted to the progeny at least of the succeeding two self-bred generations. Since Soviet authors²⁻⁴⁾ have already pointed out that successive grafting for several generations is effective in obtaining graft hybrids, the present author has also tried to examine the availability of this method by using *Capsicum annuum* L.

The present paper deals with some results obtained in the progeny of a grafted plant for three successive generations, in comparison with the results previously observed in the progeny of the grafted plant for one generation.

Material and Methods

The material used in this experiment was the progeny of GY₀-13 plant, which was one of the Yatsubusa plants grafted on Spanish Paprika in 1954. A diagnostic character of Spanish Paprika appeared in two fruits of the GY₀-13 plant grafted on the stock of Spanish Paprika, but not in fruits of the Y₀-13 plant used as a control (Figs. 1 and 2). The details about the GY₀-13 and the Y₀-13 plant were described in the preceding paper.

Successive grafting was carried out as follows: Some of the seeds obtained from one of the transformed fruit on GY₀-13 were sown in 1955. One of the seedlings bearing five leaves was grafted again on a stock of Spanish Paprika, which was at a stage of development more advanced than that of the scion plant. In 1956, an offspring of the secondly grafted Yatsubusa plant was grafted once more on Spanish Paprika. In this case, two scions were grafted at the same time on a single plant (Fig. 3). Thus, Yatsubusa was repeatedly grafted on the same variety, Spanish Paprika, for three generations (Fig. 3). In these cases, cleft grafting was applied throughout. The grafted plants were cultivated in flower-pots, and the seeds obtained from them were sown in an experimental field. New leaves formed on the grafted Yatsubusa were removed as soon as possible.

In order to avoid cross pollination, the first grafted plant was isolated in a greenhouse, and each flower of the second and the third grafted plants was covered with a bag, the open end of which was carefully plugged with cotton (Fig. 4).

The abbreviations used in this paper are as follows: The figures attached to G indicate the number of successive grafting, and those attached to Y denote the number of generation; e.g. G₃Y₀ means Yatsubusa plant grafted for three generations, and G₃Y₁ the first self-bred generation of G₃Y₀, and so on.

* Reported at the 32nd General Meeting of the Genetics Society of Japan (1960).

** Biological Laboratory, Faculty of General Education, Tokyo University of Agriculture and Technology, Fuchu, Tokyo, Japan.



Fig. 1. Transformed fruits (shown by arrows) on G_1Y_0 -13 plant, which was grafted on Spanish Paprika. Fig. 2. Standard fruits on the control plant. Y-13 was grown from the remaining part of the Yatsubusa. Fig. 3. G_3Y_0 -13 plant grafted on Spanish Paprika for three successive generations. St, stock; Si, scion.

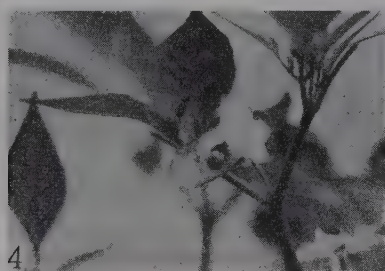


Fig. 4. A flower covered with a bag to avoid cross pollination.

Results

1. *Results obtained from successive graftings for three generations (G_3Y_0):* Transformed fruits were formed not on G_2Y_0 plant but on G_3Y_0 plant (Figs. 3 and 5).

These fruits were found together with the standard ones of Yatsubusa on a single plant. There were several fruits having valleculeae (one of the typical characters for Spanish Paprika) on the top (Fig. 5).

Seeds from a conspicuously transformed fruit of G_3Y_0 plant were conserved for the test of the first self-bred generation. The transformed fruit is indicated with an arrow in Fig. 5.

2. *Results obtained in the first self-bred generation (G_3Y_1):* The seeds conserved for this generation were sown in a nursery bed on March 1st, 1957. The young plants were then transplanted to the experimental field.

Fruits of various shapes appeared in this generation. They may be divided into ten groups as shown in Table 1. Many of them had the valleculeae on the fruit top as the mother fruit shown in Fig. 5 (Fig. 6). These valleculeate fruits appeared together with cuspidal ones on a single plant just as in the previous generation (Fig. 7). The rate of occurrence of such valleculeate fruits was 7.3 per cent (86 out of 1181 fruits). Plants bearing the valleculeate fruits appeared at as much as 91.4 per cent (32 out of 35 plants). In a single plant, 17.5 per cent of the fruits had the valleculeae. This was the highest value in the family. The details are shown in Table 1.













Fig. 5. A typical transformed fruit (shown by an arrow) on G_3Y_0 -13 plant, seeds of which were used for the test of the first self-bred generation, G_3Y_1 . Fig. 6. Valleculeate fruits produced in the G_3Y_1 -13 family. Fig. 7. Transformed fruits among the standard ones on a single plant. Fig. 8. Mother fruits used for the test of the second self-bred generation, G_3Y_2 ; from left to right, G_3Y_1 -13B, G_3Y_1 -13C, G_3Y_1 -13A.

3. *Results obtained in the second self-bred generation (G_3Y_2):* Out of the valleculeate fruits formed on G_3Y_1 , three were chosen for the test of the second self-bred generation. These three fruits were designated as G_3Y_1 -13A, G_3Y_1 -13B and G_3Y_1 -13C, respectively (Fig. 8). The seeds obtained from them were sown separately in three plots of the nursery bed on March 1st, 1958.

In this generation, the results were quite similar to those obtained in the preceding generation, that is, various transformed fruits, including valleculeate ones, were observed on the plants of all three families (Table 2). Several valleculeate fruits are shown in Figs. 9, 10, 11 and 12. These fruits appeared at the rate of 3.4 per cent in the family G_3Y_2 -13A, 11.6 per cent in G_3Y_2 -13B, 2.2 per cent in G_3Y_2 -13C, namely

Table 1. Results in the first self-bred generation after successive grafting for three generations; G₃Y₁.

No. of plant	Fruit types and number of fruits										Total number	% of occurrence of J-type fruits	
	A	B	C	D	E	F	G	H	I	J			
													
1	30							1		1	3	35	8.6
2	38	2	2	3		1			1	5	11	63	17.5
3	31		1	1			19	2			1	55	1.8
4	42		1	3		3	1			2	4	56	7.1
5	14	2	2			1					1	20	5.0
6	17	3	1	1			2			2	5	31	16.1
7	26		3	3			5			3	7	47	14.9
8	43			1							1	45	2.2
9	26	1				3	1			2	2	35	5.7
10	3											3	0.0
11	30	1		1	2		1	1		2	1	39	2.6
12	23					3	5			1	2	34	5.9
13	14					1	3			2	2	22	9.1
14	33	1				2					1	37	2.7
15	21	2		2		2				1	4	32	12.5
16	41			1							1	43	2.3
17	19	1		6		4			1	1	2	34	5.9
18	27						1				1	29	3.4
19	14					1			1		1	17	5.9
20	34						1			1	2	38	5.3
21	45					3				1	1	50	2.0
22	13					2					1	16	6.3
23	88	2	2	1	3					10	5	111	4.5
24	2								1		4	7	(57.1)*
25												0	0.0**
26	2											2	0.0
27	5											5	0.0
28	27										4	31	12.0
29	18									1	3	22	13.6
30	21		1		1						3	26	11.5
31	14	1		1			2	1		1	3	23	13.0
32	24	2	2	4	2					4	3	41	7.3
33	24		1	2							1	28	3.6
34	22		1								1	24	5.0
35	31		4							7	2	44	4.5
36	27		1			4	1				3	36	8.3
Total	889	18	22	30	8	30	43	8	47	86		1181	
%	75.3	1.6	1.8	2.5	0.7	2.5	3.6	0.7	4.0	7.3			

* Exceptional value due to poor yields.

** Withered plant.



Figs. 9, 10, 11 and 12. Transformed fruits observed in the G_3Y_2 -13 families.

Table 2. Results in the second self-bred generation after successive grafting for three generations; G_3Y_2 .

Fruit	No. of plant	Fruit types and number of fruits								Total number
		A	B	C	D	E	F	G	H	
G_3Y_2 13A	21	299	0	3	2	0	1	6	11	322
G_3Y_2 13B	6	104	0	3	1	4	0	2	15	129
G_3Y_2 13C	13	177	2	0	0	0	0	2	4	185
Total	40	580	2	6	3	4	1	10	30	636
%		91.2	0.3	0.9	0.5	0.6	0.2	1.6	4.7	

4.7 per cent on an average.

The rate of occurrence of the plants bearing vallecuate fruit was 28.6 per cent (6 out of 21 plants) in the family G_3Y_2 -13A, 83.3 per cent (5 out of 6 plants) in G_3Y_2 -13B, 30.8 per cent (4 out of 13 plants) in G_3Y_2 -13C, and then 37.5 per cent (15 out of 40 plants) on an average. The highest rate, at which the vallecuate fruits were found on a single plant was 25.0 per cent, in this generation.

4. Results in the first and second self-bred generations of *Yatsubusa* grafted only for one generation (G_1Y_1 , G_1Y_2): In order to examine whether the progeny of G_1Y_0 differs from that of G_3Y_0 or not, 21 seedlings which were not used for successive

Table 3. Results in the second self-bred generation of the plant grafted for one generation; G_1Y_2 .

No. of plant	Fruit types and number of fruits								Total number
	A	B	C	D	E	F	G	H	
1	5	2	1		1				9
2	2	3	2						7
3	12					1			13
4	9				3				12
5	9		1						10
6	4							1	5
7	6								6
8	7								7
9	9	1							10
10	9			1	3			2	15
11	16								16
12	3	11		1					14
13	12	2							14
14	9								9
15	18	4		3					25
16	2								2
17	12								12
18	3		1		1	1	1	1	8
19	7	1		1					9
20	2	2				1			5
21	7								7
22	1	7		1	1		1		11
23	10	1							11
24	11	1							12
25	9								9
Total	194	34	5	7	9	3	2	4	258
%	75.1	13.2	1.9	2.7	3.5	1.2	0.8	1.6	

graftings were kept on cultivation in 1955. All 192 fruits obtained from them showed the standard shape of Yatsubusa. Accordingly, the diagnostic character of the transformed mother fruit (Fig. 1) was not transmitted to the progeny. A few vallecuate fruits appeared, however, in the second self-bred generation G_1Y_2 at the rate of 1.6 per cent (4 out of 258 fruits). The details are shown in Table 3. The plants bearing such fruits appeared at the rate of 12.0 per cent (3 out of 25 plants). The highest rate of occurrence of transformed fruits on a single plant was 13.3 per cent. These values were not so high as those in G_3Y_1 and G_3Y_2 . The average rate of occurrence of vallecuate fruits in G_3Y_1 and in G_3Y_2 is 7.5 times as much as that in G_1Y_1 and in

Table 4. Comparison of the results obtained by grafting for one generation (G_1) with those by successive grafting for three generations (G_3).

	Generation	Fruit types and No. of fruits										Total number	Number of plds.	Number of plds. with J-type fruits/total
		A	B	C	D	E	F	G	H	I	J			
G_1	Y_1											192	21	0/21
	%	100	0	0	0	0	0	0	0	0	0			0%
	Y_3	194	34	5	7	9	3	0	0	2	4	258	25	3/25
	%	75.1	13.2	1.9	2.7	3.5	1.2	0	0	0.8	1.6			12.0%
	$\frac{Y_1+Y_3}{2}$ %	87.55	6.6	0.95	1.35	1.75	0.6	0	0	0.4	0.8			6.0%
G_3	Y_1	899	18	22	30	8	30	43	8	47	86	1181	35	32/35
	%	75.3	1.6	1.8	2.5	0.7	2.5	3.6	0.7	4.0	7.3			91.4%
	Y_2	580	0	2	6	3	0	4	1	10	30	636	40	15/40
	%	91.2	0	0.3	0.9	0.5	0	0.6	0.2	1.6	4.7			37.5%
	$\frac{Y_1+Y_2}{2}$ %	83.25	0.8	1.05	1.7	0.6	1.25	2.2	0.45	2.8	6.0			64.45%

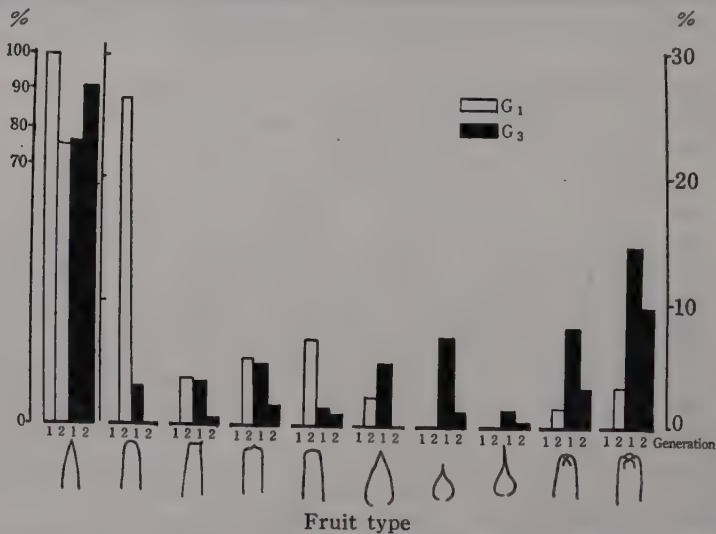


Fig. 13. A Histogram showing the rates of occurrence of various fruit types in G_1 and G_3 . G_1 : progeny of the plant grafted for one generation. G_3 : progeny of the plant obtained by successive grafting for three generations.

G_1Y_2 . And the average rate of occurrence of plants bearing transformed fruits in G_3Y_1 and in G_3Y_2 is 10.7 times as much as that in G_1Y_1 and G_1Y_2 .

The results of the present experiments are shown collectively in Table 4 and Fig. 13. These indicate that successive grafting is quite effective in inducing transformation of the fruit shape, and this corresponds with the view of Soviet authors on the effect of successive grafting.

Discussion

The results obtained in the present experiments and the previous ones are discussed here as a whole. First, the cause of the variation in fruit shape induced by grafting is considered.

The variation in fruit shape such as observed in the experiments might be thought to have occurred as a result of (1) natural crossing, (2) natural mutation, (3) genetic impurity of the test plants, or (4) chimera formation. The dubieties may be clarified by the following reasons:

(1) As described in the preceding paper, the fruit shape and fruiting habit in F_1 plants, which were obtained by crossing between Yatsubusa and Spanish Paprika, are essentially different from those found in grafted plants and their progenies. Therefore, it may be reasonable to conceive that the variation observed on the grafted plants and their progenies was not induced by natural crossing. (2) The results of the second experiment described in this paper may provide an answer to the second question. The rate of variation tends to increase after successive grafting and far exceeds the level of mutation rates accepted in common. Besides the shape of transformed fruit obtained by grafting was shown to be intermediate one between Yatsubusa and Spanish Paprika. These facts indicate that the variation in fruit shape was not caused by natural mutation. (3) As to the third question, possibility of genetic impurity of the test plants has been excluded by careful control experiments as described in the preceding paper. (4) All the shoot of the scions or the stocks, containing those bearing transformed fruits, were developed from the buds of their own which had existed at the time of grafting. No callus bud was formed in the region of grafting. Chimera or tissue mixture, therefore, could not arise in these experiments.

It seems that these facts show inapplicability of the four causes mentioned above.

On the other hand, remarkable features of graft hybrids are summarized as follows from the works of I. E. Glushchenko^{2,5}), Luzhitsa Glavinich⁶), B. D. Fajnbron⁷) and others.

(a) The change of characters induced by grafting usually appears in the first self-bred generation or later. But, sometimes it occurs on the grafted plant itself, either on the scion or on the stock. (b) Segregation of characters is observed in the progenies of a graft hybrid as well as in sexual hybrids. (c) A peculiar phenomenon observed in graft hybrids is the fact that characters of the scion and the stock appear in a mosaic pattern within an individual of the grafted plant or its progenies. (d) The hybrid obtained by grafting shows a new form combining the characters both of the scion and the stock plant. (e) In some cases, it happens that quite new characters never shown by the both components appear on a graft hybrid. (f) By means of grafting, it is possible to change a form which is a recessive character into another form which is a dominant one and *vice versa*. (g) Both of the allelic characters of the scion and the stock plant may appear in the first self bred generation of the graft hybrid. (h) In the progenies of a graft hybrid, the dominant character may, sometimes, occur within a plant having the recessive character predominantly. (i) Heterosis may arise in graft hybrids as well as in sexual hybrids. (j) It is possible to heighten the degree of variation by means of successive grafting repeated for several generations.

The results of the experiments conducted by the present author on red pepper

are in good correspondence with some of the facts mentioned above: e.g. (a), (c), (d) and (j).

According to the results of the crossing between Yatsubusa and Spanish Paprika described in the preceding paper, the cuspidal shape of the fruits in Yatsubusa is dominant over the vallecule bell-form in Spanish Paprika, the habit of fruiting "pendulous" (in Spanish Paprika) over "upright" (in Yatsubusa), and single character of bearing a single fruit on each node (in Spanish Paprika) is dominant over the character of bearing several fruits in a cluster (in Yatsubusa).

In the two grafting experiments, the recessive vallecule form was expressed by transforming the dominant fruit shape of Yatsubusa plant which was grafted on Spanish Paprika. This is consistent with the statement (f). Such a transformation was seen in a few fruits on a plant and the other remained unchanged, so that mosaic individuals showing both dominant and recessive characters appeared in this case. This fits in with the item (c). In the progenies from the transformed fruit having the recessive character, mosaic plants bearing dominant and recessive fruits appeared together with the plants having only the standard fruits of dominant character (Fig. 14). This seems to be different from the segregation in the sense of (b). But the

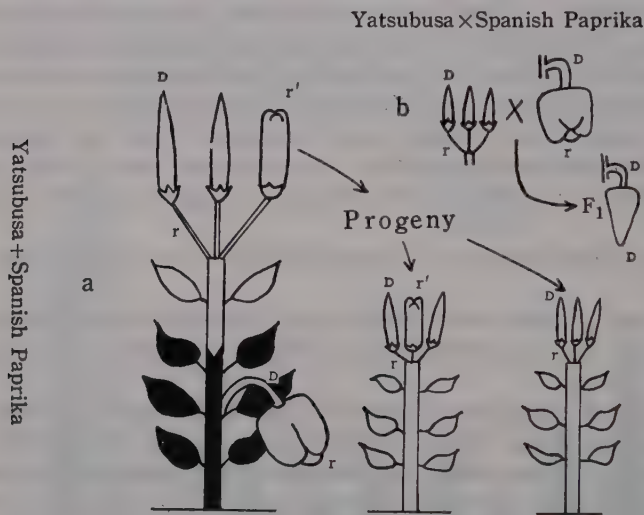


Fig. 14. Schematic illustration showing the simultaneous appearance of the recessive and dominant characters. a, in the case of sexual hybrid; b, in the case of graft hybrids. D, dominant character; r, recessive character; r', recessive character expressed by transforming the dominant character.

fact of expression of the recessive character in the grafted plants and their progenies is consistent with (g) and (h). In the other allele, i.e. the habit of fruiting "upright", which represents a recessive character of Yatsubusa was scarcely affected by the dominant one "pendulous", since such a case has been observed only in two among thousands of fruits hitherto obtained in the progenies of the grafted plants. Accordingly, these two allelic characters relating to the fruit shape and the fruiting habit seem to be independent from each other in respect of the conversion between the

dominant and the recessive character. The phenomenon of heterosis such as stated in (i) was not found in the experiments.

To sum up, the results of the two grafting experiments seem to show a close similarity to the remarkable features of graft hybrids as was pointed out by Grushchenko and others. Therefore, it may be said that changes in hereditary characters can be induced by means of grafting or successive grafting.

Summary

1. An additional experiment was made with two varieties of *Capsicum annuum* L. to ascertain the possibility of obtaining graft hybrids by means of successive grafting.

2. The diagnostic character of the fruit shape in Spanish Paprika, which was used as the stock plant, appeared in several fruits formed on the scion plant, Yatsubusa, which had been successively grafted on Spanish Paprika for three generations. This character was transmitted to the first and second self-bred generations.

3. The rate of occurrence of transformed fruits in the progeny obtained through successive grafting was much higher than that found in the first and second generations of the plant which was grafted for only one generation.

4. Discussion was made on the cause of hereditary transformation observed in a series of grafting experiments.

The author wishes to express his cordial thanks to Prof. K. Fukumoto for his helpful advice throughout the experiments, and also to Dr. K. Hayashi, Professor of Tokyo University of Education, for his help in preparing the manuscript.

References

- 1) Yagishita, N., Bot. Mag. Tokyo 74: 122 (1961).
- 2) Glushchenko, I. E., Vegetative Hybridization in Plant, Moscow (1948).
- 3) Hazina, E. P., Dokl. BASHNIL 6: 9 (1952).
- 4) Tsitsin, N. B., and Nazarova, M. Z., Izvestiya Akad. Nauk SSSR. Ser. Biolog. 1: 20 (1953).
- 5) Glushchenko, I. E., Dokl. BASHNIL 1: 13 (1959).
- 6) Luzhitsa Glavinich, Agrobiologiya 1: 86 (1956).
- 7) Fajnbron, B. D., Tr. Instituta Genetiki AN. SSSR. 20: 211 (1953).

摘 要

柳 下 登： トウガラシのつぎ木雑種に関する研究 II. 反復つぎ木による果形の変異と子孫への影響

トウガラシ (*Capsicum annuum* L.) の 2 品種を用いて、反復つぎ木によるつぎ木雑種の追試験をおこなった。

つぎ木を 3 代くりかえしたつぎ穂植物 (ヤツブサ) の果実に、台木植物 (アマトウガラシ) の果形の特徴があらわれた。この特徴は、その果実からえられた第 1 および第 2 の自家受精世代に伝えられた。変異果の出現率は、1 代しかつぎ木しなかった同一材料の第 1 および第 2 の自家受精世代のそれよりも、かなり高くなっていた。

本研究の第 1 報と第 2 報とに記した実験結果を総括して、ここで論議した。(東京農工大学一般教育部生物学研究室)

Culture Experiments on the Relation of Lake Bacteria to Lake Type

by Shizuo SUZUKI*

Received July 9, 1961

Many attempts to classify the lakes on various limnological bases have been carried out during past three decades¹⁻⁹). These attempts consisted of quantitative measurements of plankton, bottom fauna, bacteria and so others at a given period, and they have indicated only the inherent capacity of lake to support life. It has been desired, however, to classify the lakes more dynamically. As to the relation of bacteria to lake typology, the writer has made some experiments on the physiological specificity of bacteria of different lake types.

Experimental Method

The sampled lake waters and bottom muds of different lake types were taken into sterilized test tubes and brought back to the laboratory, and then sterilized by autoclaving as soon as possible. The bacteria samples from the lake waters and muds of different lake types, were diluted respectively to the concentration of 10-50/ml. and 10-100/ml. with sterilized lake water. The equal amount of bacteria was added into the sterilized sample waters or muds, and incubated at 30° for 24 hours or more. The test tubes of same size were used to prevent the error for multiplication of periphytic bacteria. After incubation the quantitative studies were made by the plate culture method using the Henrici's medium, and the rates of multiplication of bacteria were compared.

Experiments and Results

1. *Multiplicating rates of water bacteria*: The sample waters were taken from seven lakes belonging to eutrophic, dystrophic or acidotrophic type. The bacteria of Lake Haruna, Fudoike and Ichinuma were used as the inoculating indicator representing the bacterial flora of eutrophic, acidotrophic and dystrophic lake type, respectively.

The rates of multiplication of inoculated bacteria differed with the differences of lake types as well as with the kinds of inoculating bacteria. The bacteria of eutrophic lake could easily multiply in the waters of eutrophic and mesotrophic lakes, but not in the waters of acidotrophic and dystrophic ones. On the other hand, the bacteria of acidotrophic lake increased rapidly in the waters of acidotrophic and dystrophic lakes, but not in that of eutrophic lakes. The same tendency was observed in the case of bacteria of dystrophic lake.

These results indicated that the water bacteria adapted physiologically to the specific nature of lake water in which they lived. Moreover, the physiological nature of them showed considerable variation within the same lake type. For example, Lake Ichinuma and Kaminokoike, which belonged to dystrophic type, were characterized by high contents of humic substances. According to physico-chemical specifi-

* Department of Microbial Chemistry, Faculty of Pharmacy, Tokyo College of Science, Kagurazaka, Tokyo, Japan.

Table 1. Multiplication of bacteria in the lake waters of different lake types (n/mL).

Lake type		Eutrophic	Acidotrophic	Dystrophic
Lakes from which inoculating bacteria were sampled		Harunako	Fudoike	Ichinuma
Initial amount of bacteria		0.005×10^4	0.2×10^2	0.3×10^2
Harmonic	Shinseiko (pH=7.3)	251 "	0.5 "	0
	Harunako (8.3)	291 "	11 "	—
Acidotrophic	Katanuma (1.9)	0	0	0
	Fudoike (2.9)	0.0001 "	1900 "	0.02 "
	Rurinuma (4.4)	0.001 "	2150 "	— "
Dys-trophic	Kaminokoike (4.8)	0	172 "	14 "
	Ichinuma (5.0)	228 "	244 "	2 "

Table 2. Multiplication of bacteria in the lake waters of eutrophic, mesotrophic and oligotrophic lakes (n/mL).

Lakes from which inoculating bacteria were sampled		Shinseiko	Harunako	Yamanakako
Initial amount of bacteria		0.04×10^3	4×10^4	0.005×10^4
Eutrophic	Shinseiko	44×10^3	112×10^4	64×10^4
	Nishiyaginuma	54 "	136 "	52 "
Mesotrophic	Harunako	174 "	780 "	61 "
	Yamanakako	68 "	20 "	116 "
	Kagamiike	157 "	316 "	15 "
Oligotrophic	Unagiike	—	134 "	39 "
	Rokukannon Miike	28 "	30 "	3 "
	Oonamiike	13 "	59 "	0.3 "

ty, the lake water of the latter is more disharmonic than that of the former, and the multiplying rate of bacteria was comparably scarce in the water of the latter. The same tendency was also observed in acidotrophic lakes. The multiplication of bacteria could not be seen in the strong acidic water of Lake Katanuma even in case of bacteria of acidotrophic type.

2. *Multiplicating rates of mud bacteria:* The rates of multiplication of mud bacteria differed with mud samples as well as with inoculating bacteria. The growth of mud bacteria of harmonic lake was very restricted and it was limited only in muds of harmonic lakes.

Table 3. Multiplication of bacteria in bottom muds of different lake types (n/ml.)

Lake type		Mesotrophic	Acidotrophic	Dystrophic
Lakes from which inoculating bacteria were sampled		Yamanakako	Fudoike	Kaminokoike
Initial amount of bacteria		0.02×10^4	0.08×10^3	0.01×10^3
Eu- and mesotrophic	Yamanakako	8570 "	15600 "	12300 "
	Kagamiike	8040 "	700 "	5376 "
	Shinseiko	6200 "	2100 "	9810 "
Acidotrophic	Fudoike	0.009 "	0.4 "	0
	Bentennuma	0.002 "	0.05 "	0.2 "
	Bishamonnuma	0.003 "	0.06 "	0.5 "
Dystrophic	Kaminokoike	0.003 "	—	310 "
	Ichinuma	0.2 "	290 "	—
	Nagaike	0.04 "	150 "	24 "

Table 4. Multiplication of bacteria in bottom muds of eutrophic, mesotrophic and oligotrophic lakes (n/ml.)

Lakes from which inoculating bacteria were sampled		Shinseiko	Yamanakako	Unagiike
Initial amount of bacteria		0.5×10^2	1×10^2	0.4×10^2
Eutrophic	Yanaginuma (pH: 5.8)	118×10^2	728×10^2	113×10^2
	Jimushonuma (7.6)	146 "	1040 "	390 "
	Choko (4.8)	0 "	2 "	35 "
	Tadenoumi (4.8)	2 "	— "	14 "
Mesotrophic	Yunoko (4.3)	1 "	0 "	0.4 "
	Inako (4.4)	5 "	3 "	2 "
	Kirigomeko (5.6)	1 "	5 "	0.4 "
	Karigomeko (3.6)	2 "	2 "	0.1 "
	Kagamiike (4.8)	8 "	2 "	123 "
	Yamanakako (7.1)	3640 "	15200 "	280 "
	Kawaguchiko	1 "	77 "	10 "
	Shojiko	1 "	26 "	1 "
	Ono (Akagi) (5.0)	10 "	63 "	16 "
Oligotrophic	Unagiike (5.0)	3 "	42 "	12 "
	Kono (Akagi) (5.0)	5 "	28 "	9 "
	Rokukannon Miike (4.9)	1 "	28 "	0.8 "
	Towadako (7.3)	820 "	2480 "	— "

On the other hand, the mud bacteria of acidotrophic and dystrophic types could only increase in the muds of harmonic and dystrophic lakes, but not in acidotrophic

one. The muds of acidotrophic lakes were constituted mainly of mineral sediment, such as sulfur, iron or manganese sediment. These mineral sediments may be harmful for the growth of bacteria.

The differences of the multiplication of bacteria were very conspicuous within harmonic lakes. One of the most important causes for the differences of multiplication of bacteria was the pH value of sample mud. The bacteria multiplied to the amount, approximately 300-7280 times the initial amount in Lake Towadako, Yamanakako and Jimushonuma. The pH values of the bottom muds of these lakes were neutral or slightly basic (pH: 7.1-7.6). From these results it may be said that the rate of multiplication of bacteria in bottom mud of harmonic lake type relates closely to pH value of muds, rather than the eutrophication of lakes, so far as the obtained results are concerned.

Summary

The rates of multiplication of lake bacteria were studied in some Japanese lakes. The physiological natures of water bacteria differed with lake types. Bacteria seemed to have been adapted to the specific nature of lake in which they lived. The multiplication of bacteria was very high in eutrophic and mesotrophic lakes, while it was scarce in oligotrophic one.

The lake type had not so close a relation to the multiplication of mud bacteria as to that of water bacteria. The activity of mud bacteria was influenced remarkably by pH value of bottom deposits.

The writer wishes to express his cordial gratitude to Prof. H. Indoh under whose guidance this research has been carried out. Also he thanks Dr. S. Ichimura and Prof. T. Tatsuno for their valuable suggestion and encouragement.

References

- 1) Naumann, F., Arch. f. Hydrob. **20**: 191 (1929).
- 2) Thienemann, A., ibid. Suppl. 8: 205 (1931).
- 3) Ueno, M., Chikyu **17**: 111 (1932).
- 4) Yoshimura, S., Bull. Otuka Geogr. Soc. **2**: 159 (1933).
- 5) —, Jour. Assoc. Advance Sci. **11**: 170 (1936).
- 6) Miyadi, D., ibid. **12**: 418 (1937).
- 7) Hada, Y., ibid. **13**: 431 (1938).
- 8) Yoshimura, S., Sci. Rep. Tokyo Bunrika Daigaku, Sec. C **2**: 63 (1938).
- 9) Suzuki, S., Jap. Jour. Limn. **21**: 58 (1960).

摘 要

鈴木 静夫： 培養実験による湖沼細菌と湖沼型の関係

湖水および湖沼の特性を細菌の繁殖力によって推定する方法の基礎実験として、二、三の観察を行なった。採集した湖水の水を滅菌し、この中に湖沼型を異にする湖水から分離した細菌を入れ、一定条件の下で細菌の繁殖を観察すると、湖沼型によって細菌の増殖率が異なる。これは、各型の湖沼に棲息する細菌の性質が異なることを示している。調和湖のうちでも、富栄養型、中栄養型、貧栄養型によって細菌の増殖のようすが異なる。

湖泥の場合にも、湖沼型によって同様の差異が見られ、いずれも調和湖の泥で細菌の繁殖が良好であった。これは湖泥の pH 値と関係しているものと思われる。（東京理科大学薬学部微生物化学教室）

Aneusomaty in the Leaves of Diploid *Petunia*

by Shin TAKEHISA*

Received August 18, 1961

Duncan (1945¹) reported that the somatic chromosome number within root-tips of *Paphiopedilum Wardii* fluctuates from cell to cell around the diploid number, and proposed the term "aneusomaty" for this phenomenon. This phenomenon, on the other hand, has been termed "chromosome mosaic" by Fankhauser (1945²). The occurrence of variable chromosome numbers in somatic tissue within individual plants has been reported in a number of plant families (Snoad, 1955³), Hegwood and Hough 1958⁴), Kato 1960⁵)). In *Petunia*, Levan (1937⁶)) observed the indication of aneusomaty in his F₂ individuals derived from diploid-tetraploid cross, but he did not pay any particular attention to it.

Table 1. Chromosome class distribution in the aneusomaty individual of diploid *Petunia hybrida*.

Leaf (preparation)	Chromosome class										Total number of observed cells
	14(2n)	16	21(3n)	22	23	24	25	26	27	28(4n)	
A	19	1	2	1	5	5	1	4	1	1	40
B	9		1	1	2	2	4	1			20
C	7										7
D	8										8

Examining the somatic chromosomes of *Petunia* by the leaf smear technique with the Feulgen stain (Sullivan, 1947⁷)) the author found the aneusomaty in an individual within diploid (2n=14) plants of *Petunia hybrida*, commercial name "Snow Ball" (Table 1 and Figures). A series of examination was undertaken on different young leaves taken from several stems of the plant. As shown in Table 1, about half of observed cells in the leaf A and B had increased chromosome numbers ranging from triploid to tetraploid number. The leaf C and D did not show any change of chromosome number so far as the present examination is concerned. This result indicates that the phenomenon of aneusomaty in this plant has not taken place in every leaf. There was morphologically no difference between the aneusomaty individual and the plants with normal chromosome number.

Many reports on the occurrence of aneusomaty in a number of plant families have indicated that the aneusomatic condition is apparently due to mitotic abnormalities such as split spindle, kinetochore pairing in somatic cells, and somatic bridges (Snoad, 1955³), Miduno and Yamazaki, 1952⁸), Hegwood and Hough, 1958⁴), Nielsen and Nath, 1961⁹)). Further, the aneusomaty found in wheat (Love, 1938¹⁰)), *Hymenocallis calathinum* (Snoad, 1955³)), apple (Hegwood and Hough, 1958⁴)), and *Agroelymus* (Nielsen and Nath, 1961⁹)) suggests that the potentiality to produce aneusomaty is

* Botanical Institute, Faculty of Science, Hokkaido University, Sapporo, Japan.

heritable or has relation to hybridity. The present case of aneusomaty represents the sporadic occurrence of aneusomaty in the leaves of one individual. The species *Petunia hybrida* has originated from interspecific hybridization since 1937 or so (Stout, 1952¹¹). Therefore it is considered that the present case of aneusomaty may have a background of genetical potentiality to produce aneusomaty and have a correlation to the hybrid origin of the species. However, the existence of genetical potentiality to produce aneusomaty is nothing but a remote cause of aneusomaty, and this cannot explain the manner through which these variable chromosome numbers have arisen. In connection with this point, there is a report that the mitotic abnormalities such as sticky bridges could be induced by genetical endogenous factors, i.e. the mitotically abnormal condition observed in the young leaf meristem of *Ginkgo biloba* L. is due to some endogenous substances, which become effective when amounted up over a certain threshold concentration during a specified growth period (Tanaka *et al.*, 1952¹²). On the contrary, in the present case, multinucleate cells which suggest the pre-existence of split spindle and cells with subdiploid chromosome numbers probably resulting from kinetochore pairing or somatic bridges were not observed. The lack of chromosome numbers from $2n$ to $3n$ only with one exception, if it is true, may also suggest that a gradual stepwise increase of chromosome number is improbable. These facts suggest that other mechanisms than mitotic abnormality may be responsible for the occurrence of aneusomaty in the present case.

From the fact that the aneusomatic chromosome numbers are concentrated to the range from triploid to tetraploid, two alternative mechanisms may be assumed by which the aneusomaty in this diploid *Petunia* originated. The one is that the endoreduplication (Levan and Hauschka, 1953¹³) of chromosome complement took place initially, and then the elimination of certain members of chromosomes occurred. The other is that the endoreduplication of variable number of chromosomes, not of whole chromosome complement, occurred once or sometimes at an earlier stage of development, possibly accompanied by a fluctuation of numbers through successive mitotic divisions. The latter mechanism may include the case in which the duplication of chromosome occurs differently among constituent genomes.

Since, however, the observation of whole mitotic cycle was not attempted in the present paper, which will provide with informations giving clue to the really operative mechanisms of the aneusomaty in the present case, decisive conclusion must await further studies.

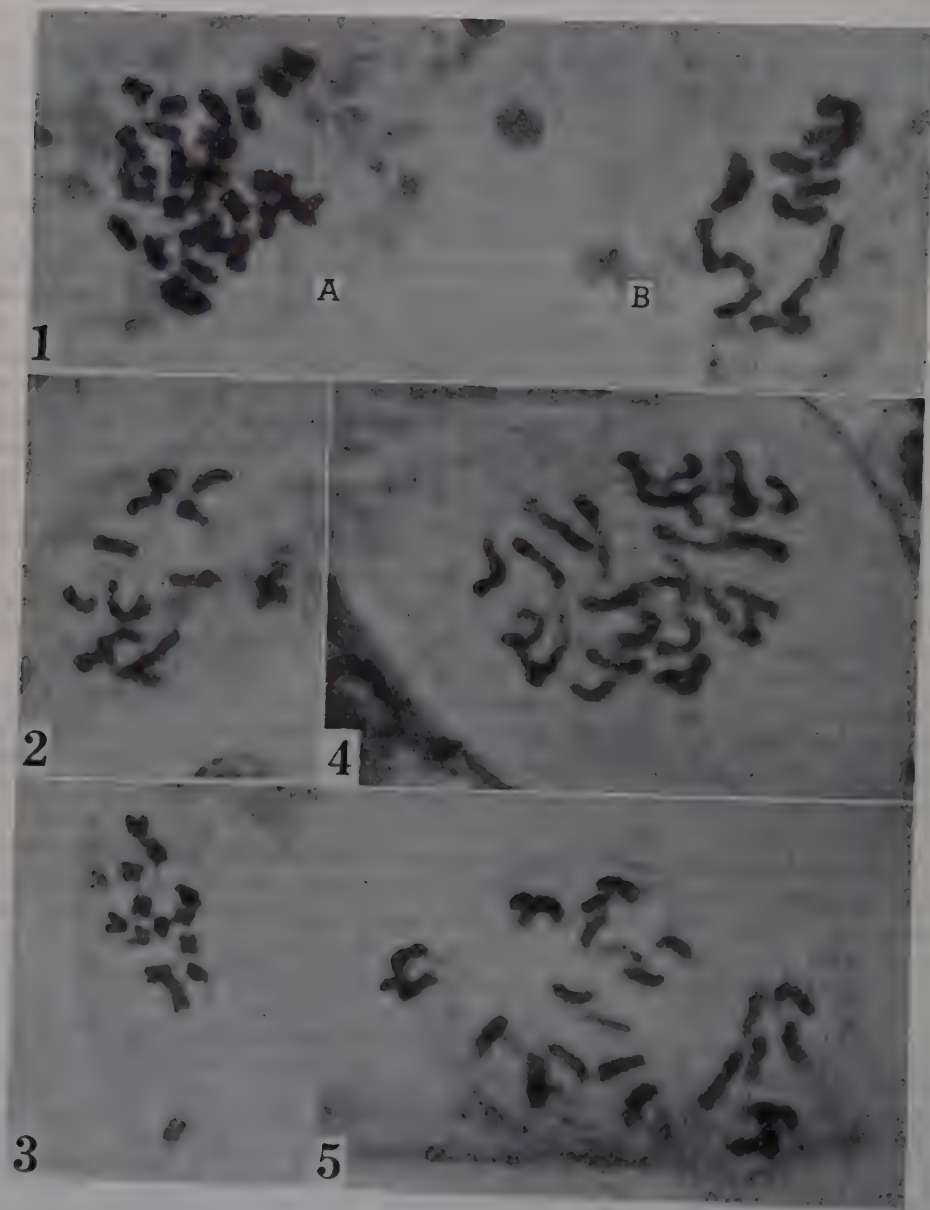
The author wishes to express his gratitude to Professor Hajime Matsuura for his suggestion and criticism during the course of this study. The gratitude is also due to Professor Tadanori Miduno, Keio University, for his kind criticism.

References

- 1) Duncan, R. E., Amer. J. Bot. 32: 506 (1945).
- 2) Fankhauser, G., Quart. Rev. Biol. 20: 20 (1945).
- 3) Snoad, B., Heredity 9: 129 (1955).
- 4) Hegwood, M. P., and Hough, L. F., Amer. J. Bot. 45: 349 (1958).
- 5) Kato, Y., La Kromosomo 42-43: 1447 (1960).
- 6) Levan, A., Svensk Bot. Tidskrift 31: 1 (1937).
- 7) Sullivan, T. D., Bull. Torrey Bot. Club 74: 453 (1947).
- 8) Miduno, T., and Yamazaki, N., Jap. Jour. Genet. 27: 210 (1952).
- 9) Nielsen, E. L., and Nath, J., Amer. J. Bot. 48: 345 (1961).
- 10) Love, M., Genetics 23: 517 (1938).
- 11) Stout, A. B., Memoirs Torrey Bot. Club 20(3); Reproduction in *Petunia* (1952).
- 12) Tanaka, N., Takemasa, N., and Sinoto, Y., Cytologia 17: 112 (1952).
- 13) Levan, A., and Hauschka, T. S., J. Nat. Cancer Inst. 14: 1 (1953).

Explanation of Figures

Photomicrographs of Feulgen leaf smears of diploid *Petunia hybrida* showing aneusomaty, ca. $\times 6000$. Fig. 1. Two adjacent cells showing tetraploid (A, $4n=28$) and diploid ($2n=14$) condition each. Figs. 2, 3. A diploid cell. Fig. 4. A cell having 27 chromosomes. Fig. 5. A cell having 26 chromosomes.



摘 要

武・久・慎：二倍体ペチュニアの葉の分裂細胞における Aneusomy

葉の分裂細胞でペチュニアの染色体を調べていた際、偶然二倍体ペチュニア (*Petunia hybrida*) の園芸品種名 “Snow Ball” と呼ばれる系統のものの中の一株が、Aneusomy を示しているのを見出した。この現象はこの個体の総ての葉に共通して起こっているのではない。 $2n=14$ の正常な染色体数からの変化がほとんど $3n$ と $4n$ との間にあることから、その原因と出現機構について簡単に考察した。（北海道大学理学部植物学教室）

Micrococcus glutamicus の細胞学的研究

第6報 細胞伸長肥大効果物質について

板垣史郎*・木幡 守*・木下祝郎*

Shiro ITAGAKI*, Mamoru KOBATA* and Shukuo KINOSHITA*: Cytological Studies on *Micrococcus glutamicus*

Part VI. Investigations of Substances which have the Effects of Cell Elongation and Enlargement

1961 年 6 月 14 日受付

緒 言

前報¹⁾において、クエン酸ナトリウムあるいはリンゴ酸ナトリウムを添加したビオチン含有合成培地中において、*Micrococcus glutamicus* はいちじるしい菌体の伸長肥大をおこし、さらに、分岐をも形成することを報告した。特に、クエン酸ナトリウムを 6~10% 添加した場合、本菌はいわゆる bifid bacteria 型を呈し、これより分岐形成過程をも推定しうることを、また、リンゴ酸ナトリウムによる分岐は多分岐をも形成し、ときに二次的な分岐形成もみられることなどを述べた。

このように、本菌の teratological growth-form と物質との間には重大な関連性があると考えられるので、さらに伸長肥大分岐形成などを中心として種々の物質の影響を観察し、あらたに二、三の物質の形態におよぼす効果などを知りえたので、ここに報告する。

実験材料および実験方法

使用菌株

Micrococcus glutamicus 582 を主として用いた。

供試菌株を glucose bouillon 培地に振盪前培養し、試験培地に対し 10% の割に加え、28°C にて振盪培養をおこなった。基本培地はすでに述べたとき合成培地²⁾にビオチン 1 γ /l 添加したもので

* Tokyo Research Laboratory, KYOWA HAKKO KOGYO Co., Ltd., Tokyo, Japan. 協和醸酵工業株式会社東京研究所

あり、この基本培地に対して菌体の伸長肥大分岐誘発性などの有無を調べる目的の種々の物質を添加したものを試験培地とした。

添加した物質はつぎの四つに大別される。すなわち、1. クエン酸塩類 2. 種々の有機酸のナトリウム塩 3. アミノ酸 4. 抗生物質。

培養を経時的に採取し、pH, O.D. 値を測定し、菌体をメチレン青にて染色して形態を観察した。

結 果

1. クエン酸塩類

前報¹⁾で述べたごとく、クエン酸ナトリウムおよびリンゴ酸ナトリウムには伸長肥大分岐効果があるが、リンゴ酸ナトリウムには同時に強い溶菌作用があるので、観察が充分におこなわれがたい。この理由でここでは種々のクエン酸塩について、ナトリウム塩でみられたとき著明な伸長肥大分岐効果があるかどうかを試験した。

使用したクエン酸塩の種類と添加量および生育の関係を第1表に示した。

13種のクエン酸塩中、ナトリウム塩のごとく伸長肥大分岐効果を示めたものは、アムモニウム塩のみであり、若干その効果は劣るが、カリウム塩もかなり強い。リチウム塩では菌体の膨大化がみられた。

(1) クエン酸アンモニウム (NH_4 -citrate)

2% 添加で伸長、多細胞体が形成され、4~6% では長い bifid bacteria 型となつた。このとき、

第1表 クエン酸塩の種類と生育の関係

塩の種類	添加量%	pH	生育	塩の種類	添加量%	pH	生育
NH ₄	2	6.6	卅	Mg	2	6.8	卅
	4	8.8	+		4	8.4	卅
	6	8.8	+		6	8.0	卅
	8	8.6	+		8	8.2	卅
	10	8.2	±		10	8.8	—
Li	1	9.0	±	Ca	飽和(約 0.1%)	5.4	卅
	2	9.0	—	Zn	飽 和	8.2	—
	4	9.0	—	Sr	飽和(約 0.3%)	5.4	卅
	6	8.8	—				
	10	7.8	—	Cd	0.01 飽和	9.2	—
Na	5	9.2	±		0.02	9.2	—
	10	7.8	±		0.05	9.2	—
K	2	5.4	卅		0.1	9.2	—
	4	6.6	卅		0.2	9.2	—
	6	8.8	+		0.5	9.2	—
	8	9.0	—		1.0	9.2	—
	10	9.0	—	Ba	飽和(約0.04%)	8.8	卅
Cu	0.01 飽和	5.4	卅	Hg	0.01 飽和	5.0	卅
	0.02	5.6	卅		0.02	5.4	卅
	0.05	8.8	卅		0.05	5.4	卅
	0.1	8.8	卅		0.1	9.2	—
	0.2	9.2	—		0.2	9.0	—
	0.5	9.2	—		0.5	9.2	—
	1.0	9.0	—	Al	5	8.6	卅
					10	8.2	卅

分岐細胞も観察された。8~10% 添加では生育はまったくみられず、metachromatic granule の発現が顕著であつた。

(2) クエン酸カリウム (K-citrate)

2~4% 添加では伸長多細胞体が形成されるが、さしていちじるしいものではない。6% にいたり肥大多細胞体はいわゆるこん棒状を呈し、まれに分岐がみられる。8% 以上添加することにより生育は停止する。

(3) クエン酸リチウム (Li-citrate)

リチウム塩は生育阻害が強く、1% 添加ですでにほとんど生育を阻害する。したがって多細胞体を形成するにいたらないが、細胞そのものはかなり膨大する。

その他の塩では特記するほどの形態変化はみられないが、銅塩において形態的にやや興味ある所見が得られた。すなわち、銅塩を 0.02~0.05 飽和程度に加えると生育はかなり良く、多細胞体が形成されるが、このときの菌体は短かく、円盤状の多細胞体

となる。また、カドミウム塩はきわめて毒性が強く 0.01飽和においてすらまったく生育がみられなかった。

2. 有機酸ナトリウム塩

クエン酸ナトリウムおよびリンゴ酸ナトリウムの伸長肥大分岐効果を認めたのは、*M. glutamicus* の代謝産物そのものの形態におよぼす影響の有無という考え方からおこなった実験の結果であつた。このように、ある種の有機酸が菌形態をいちじるしく変化せしめることが認められたので、有機酸全般について検討を加えた。

(1) 飽和脂肪酸

下記のごとき13種をナトリウム塩の形で用いた。炭素数奇数のものは *iso*-カプロン酸までである、添加量はいずれも 1.5 および 10% である。

ギ酸、酢酸、プロピオン酸、酪酸、*iso*-吉草酸、カプロン酸、*iso*-カプロン酸、カプリル酸、カプリン酸、ラウリン酸、ミリスチン酸、パルミチン酸およびステアリン酸。

ミリスチン酸以上のもののナトリウム塩はいずれも粘稠または固化し、実験しえなかった。

以上13種の飽和脂肪酸は、細胞学的見地よりみれば、かなり興味ある所見を与えたものもあるが（たとえば、酢酸 5%、カプリル酸 5% 添加などで細胞質の好塩基性をいちじるしく減じ、核様物が鮮明にみられる）、伸長肥大効果としてはみるべきものがなかった。

(2) 不飽和脂肪酸

リノール酸とリノレイン酸の2種を用いたが、菌生育がみられず、菌形態も前培養時のままを保っていた。

(3) オキシ酸、ケト酸およびオキシケト酸、乳酸、ピルビン酸、レブリン酸、オキザロ酢酸およびグルタル酸の5種を用いたが、いずれもみるべき効果はなかった。ただピルビン酸 10% 添加において菌体は肥大、やや伸長し、いく分末端細胞の膨大化が認められた。

(4) 二塩基酸

二塩基酸としてつぎの5種を用いた。すなわち、シュウ酸、マロン酸、コハク酸、アジピン酸およびアゼライン酸である。

シュウ酸には著明な伸長肥大分岐効果が認められた。2~5% 添加にて末端膨大化した伸長肥大細胞が出現し、ときに分岐を形成し、また *bifid bacteria* 型を呈する。6% 添加にて細胞は小型化し、ほとんど全部の細胞は *bifid bacteria* 型を呈する。

また、アゼライン酸はかなり高度の多細胞体を形成せしめる。したがってかなり長い細胞が混在するようになる。

(5) オキシ二および三塩基酸

このなかにはリンゴ酸、クエン酸が含まれるから当然、肥大分岐効果に期待されたが、酒石酸においてやや伸長効果を認めたのみであった。対照として用いたリンゴ酸、クエン酸では前報に述べたごとく明らかな伸長肥大分岐が認められた。

(6) 不飽和二および三塩基酸

マレイン酸、フマル酸、イタコン酸およびアコニット酸 (trans) を用いた。

マレイン酸 5%、フマル酸 5% およびアコニット酸 10% 添加でやや伸長効果を認めた。しかし、上記4種の酸添加培地では、生育は良好であり、クエン酸ナトリウム、あるいはリンゴ酸ナトリウム添

加培地におけるごとく、生育は極度に不良であるが生残した若干の細胞がいちじるしく伸長肥大するのとは異なり、かなり良く生育した菌集団中に若干の伸長細胞が認められるのである。

3. アミノ酸

つぎの17種のアミノ酸を用いた。すなわち、グリシン、DL-アラニン、L-バリン、L-ロイシン、DL-iso-ロイシン、L-スレオニン、L-シスチン、L-シスチン、DL-メチオニン、L-フェニールアラニン、L-チロシン、DL-トリプトファン、L-アルギニン、L-リジン、L-プロリン、L-アスパラギン酸およびL-グルタミン酸である。

添加量はそれぞれのアミノ酸の溶解度をも考慮し多量の場合は 100 mg/ml (10%) をも用いた。シスチン、チロシンのごとく溶解度の低いものは飽和を用いた。

一般に、上記のごときアミノ酸を添加することにより生育は若干促進されるが、50~100 mg/ml のごとく多量に添加した場合は逆に生育に阻害をきたすこともある。たとえば、アラニン 100 mg/ml 添加ではかなりの阻害が認められた。またシスチン 50~100 mg/ml 添加ではかなり強く生育が阻害される。

伸長肥大分岐のごとき菌形態の変化は、すでに述べられたごとく、いちじるしい生育不良条件下でおこるものである。したがって、アミノ酸のごとき生育を促進するか、あるいは若干抑制する程度のもものでは伸長肥大効果を期待しえないのが普通である。しかし、シスチン 50 mg/ml では生育もかなり阻害され、染色性不良の小型菌に混じ、いちじるしく伸長肥大し、末端膨大化した菌体がかかり出現してくる。

アスパラギン酸は 100 mg/ml 添加において最初、多分岐で溶菌がいちじるしいことを認めたが、その後再三の試験にもかかわらず確認しえなかった。

グルタミン酸 100 mg/ml 添加ではかなり菌体伸長がみられる。末端が膨大化した細胞が多くなる。

シスチンを除いては一般にアミノ酸添加による伸長肥大効果は大きくない。

4. 抗生物質

抗生物質により誘発される long form の形成および形態変化の報告はかなり多数にのぼる。しかし一般にこのような long form は分裂阻害の結果形

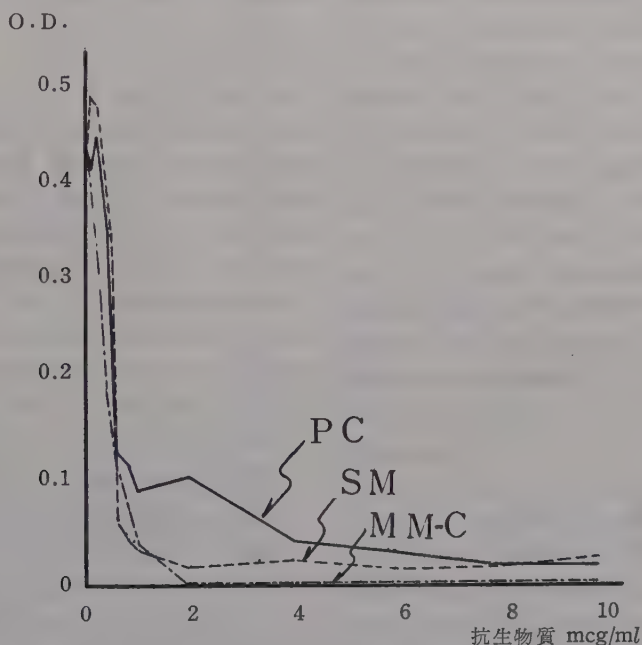
成された伸長細胞であって、*M. glutamicus* の多細胞体とは本質的に異なるものではあるが、抗生物質添加によりかなりの形態変化を期待して実験をおこなった。

使用した抗生物質はつぎの10種である。添加量は0.1, 0.5, 1.0, 5.0 および 10.0 mcg/ml とした。

ペニシリンGカリウム塩 (PC), 複合ストレプトマイシン (SM), カナマイシン (KM), クロランフェニコール (CM), クロールテトラサイクリン (AM), オキシテトラサイクリン (TM), テトラサイクリン (ACM), ザルコマイシン (SKM), マイト

(1) ペニシリン

0.6 mcg/ml (約 0.7 μ g/ml) 添加以上でいちじくしく生育を抑制し、2.0 mcg/ml まで菌体は好塩基性を増し、いちじくしい多細胞体を形成する、このさいの多細胞体は円盤状のものであって、全体の菌長に比し構成細胞数が多いのが特徴である。このときの末端細胞は膨大化し、こん棒状を呈するものが多いが、培養が2~3日経過すると特に伸長した菌体では末端細胞が反対に小型化してくるものが混在してくる。また溶菌をおこすものも多くなってくる。抗生物質添加量と生育の関係は第1図に示した。



第1図 抗生物質添加と生育 (1日培養)

PC : penicillin, SM : streptomycin, MM-C : mitomycin-C

マイシンC (MM-C) およびカルチノフィリン (CZ) である。

このうち、CM, AM (10 mcg/ml にて阻害される), TM および ACM には非感受性であった。

他の6種の抗生物質のうち、PC, SM, および MM-C の3種は細胞学的にきわめて興味ある所見をえたので、さらに添加量を細区分して実験をおこなった。特に MM-C では顕著な伸長肥大、さらに分岐、bifid bacteria 型の形成なども見られた。これらの抗生物質添加のさいの所見を以下に述べる。

このような形態変化がみられる PC 添加量は、第1図において、生育曲線が急速に下降する点上であって、生育曲線が上昇または水平化したところ、すなわち 0.2 mcg/ml 以下 また 4.0 mcg/ml 以上では形態変化は認められない。この抗生物質の添加量と生育曲線の関係は PC, SM および MM-C に共通であるばかりか、前報¹⁾におけるクエン酸ナトリウム、リンゴ酸ナトリウム添加量と形態の関係でも同様のことがいえる。

(2) ストレプトマイシン

0.2 mcg/ml 添加までは正常な生育をおこない、形態変化もみられないが、0.4~0.8 mcg/ml 添加で著明な不染部分が出現してくる。この部分はほぼ球形で、細胞の平面像の約 $\frac{1}{2}$ ~ $\frac{1}{3}$ に近い。おそらくは液胞であろう。この所見は酵母の液胞に近似している。このときの菌形態は楕円ないし短桿状で、多細胞形とはならない。SM 添加量を 1.0 mcg/ml 以上に増加すると、この液胞様構造の出現は認められなくなり、菌体全体がやや不整形となり、染色性は低下する。

(3) マイトマイシン-C

0.2~0.8 mcg/ml 添加にていちじるしい多細胞体の形成をみる。したがって菌長は長く、ときに 10 μ 以上におよぶことがある。またその幅も大となり、1.5~2.0 μ に近い。このような長大細胞では末端はこん棒状を呈するものと、その反対に末端が小さくなっているものとが混在するため、菌形は不整化する。1.0~2.0 mcg/ml 添加ではさらに伸長した菌が認められ、そのうえ、分岐も認められる一方、一般的には細胞は小変位の傾向をみせ、太いがやや短くなる。このときの菌体は PC 添加のさい生ずる円盤状に似ている。40 mcg/ml 以上添加では菌形は小さく、溶菌をおこす。2.0 mcg/ml 添加 3 日培養ではいちじるしく大きい metachromatic granule を多数形成した。

考 察

1. クエン酸塩と伸長肥大効果

クエン酸のナトリウム塩がいちじるしい伸長肥大分岐を誘発することはすでにくわしく述べたが、クエン酸の種々の塩についての家験結果はわずかにアンモニウム塩とカリウム塩においてのみナトリウム塩と同等あるいは若干弱い効果が認められたにすぎない。このほかはリチウム塩とアルミニウム塩に弱い伸長効果が認められた。しかし、他の塩類はいずれも溶解度が低いので、ナトリウム塩などと量的に比較することは困難である。銅、水銀およびカドミウム塩はいずれもきわめて低濃度で生育を停止せしめる。このことは、クエン酸塩のカチオンの影響がきわめて強く現われることをしめすものである。

形態的にみると、銅塩はかなり多細胞体形成能が強いが、伸長効果はかえってマイナスのほうに強く多細胞体のわりには全長は短かい。したがって細胞

はいわゆる円盤状となる。

2. 有機酸の構造と伸長肥大効果の関係

クエン酸ナトリウムおよびリンゴ酸ナトリウムの細胞伸長肥大、さらに分岐誘発効果の確認に引き続き、有機酸 32 種のナトリウム塩を添加し、その効果を調べた。ここに用いた有機酸のなかにはすでにその効果が確認されているクエン酸およびリンゴ酸をも含め、再確認と対照の意味をもたせた。これらのうち、あらたに伸長肥大効果を確認した物質はシュウ酸のみである。しかし、高度の多細胞体の形成、あるいは細胞集団中に伸長菌体が若干混ざるごときものとしてはピルビン酸、アゼライン酸、酒石酸、マレイン酸、フマル酸およびアコニット酸の各酸があげられる。

強い伸長肥大効果を有するクエン酸は、mono-hydroxy-tricarboxylic acid であり、リンゴ酸は monohydroxy-dicarboxylic acid である。あらたに効果の認められたシュウ酸は dicarboxylic acid であって、いずれも -COOH 基を 2 個以上有することは興味深い。しかもシュウ酸 HOOC-COOH のごとく簡単な低分子化合物にもこのような効果がみとめられたことは、構造と生理作用の関連性をしめすものごとくであるが、第 2 表にみるごとく、di- あるいは tricarboxylic acid でも大部分のものは弱い効果をしめすにすぎない。以上の結果より化学構造と伸長肥大効果を関連づけることは困難である。

3. アミノ酸添加の影響

一般に、アミノ酸をかなり多量に添加しても *M. glutamicus* は合成培地中でよく生育する。したがって、クエン酸ナトリウムなどを添加したごときとき生育不良にともなう伸長肥大細胞の出現はほとんど見られなかった。しかし、グリシン、バリン、アルギニン、アスパラギン酸およびグルタミン酸を 100 mg/ml 程度添加したときは多細胞形成の度合いがやや強まる。

シスチン添加の場合、大部分の菌は小型であるが、クエン酸ナトリウム添加に近似したごとき伸長肥大（特に末端細胞）、多細胞体が形成され、集団中に混在することが確認された。シスチン添加培地では、培養が進行するにともない、培地中のシスチンは酸化されてシステインに変化するため、溶解度が減少し析出してくる。このため生育を測定することはできなかった。顕微鏡観察によれば生育は不

第 2 表 有機酸 Na 塩の伸長肥大効果

酸	伸長肥大	多 胞 体	-COOH	-OH	-CO	= 結 合
ギ酸	N	W	1	0	0	0
酢酸	N	W	1	0	0	0
プロピオン酸	W	W	1	0	0	0
n-酪酸	N	W	1	0	0	0
i-吉草酸	N	W	1	0	0	0
β-ピロノン酸	N	W	1	0	0	0
i-カプロン酸	N	W	1	0	0	0
カプリル酸	N	N	1	0	0	0
カプリン酸	N	N	1	0	0	0
ラウリン酸	N	N	1	0	0	0
リノール酸	N	N	1	0	0	2
乳 酸	N	N	1	1	0	0
ピリン酸	W	M	1	0	1	0
レズリン酸	N	W	1	0	1	0
オキサロ酢酸	N	W	2	0	1	0
グルタル酸	N	N	1	1	1	0
シクロウン酸	S	S	2	0	0	0
マコハク酸	N	N	2	0	0	0
アジピン酸	W	W	2	0	0	0
アゼライン酸	W	M	2	0	0	0
リゼン酸	S	S	2	1	0	0
酒石酸	W	M	2	2	0	0
クエン酸	S	S	3	1	0	0
マレイン酸	W	M	2	0	0	1
フェール酸	W	W	2	0	0	1
イタコン酸	N	W	2	0	0	1
アコニット酸	W	M	3	0	0	1

(注) N：ナン, W：弱, M：中等度, S：強

良であった。

Dienes および Zamecnic⁸⁾ は *Salmonella typhimurium* と *Hemophilus influenzae* を用いてアミノ酸の形態変化および L 型菌出現を試験し、グリシンに強い L 型形成能をみとめ、また DL-メチオニン 1% 添加で *Salmonella* は large round body となり、1.5~2.0% 添加で多数の L 型コロニーが出現、また *H. influenzae* では 0.5% で生育阻害、0.5~3.0% 添加により large body と L 型コロニーの出現をみた。L(-)-フェニールアラニンも同様の効果をもっており、また 3.0% の L(-)-トリプトファン添加により *Salmonella* は生育阻害を受け、pleomorphism をしめし、少数の L 型コロニーをつくる。L(-) チロシン 1.0% 添加により *Salmonella* の生育は阻害されるが、L 型はつくらない、などの点を報告している。

このように通常は栄養成分として働らくアミノ酸もある種の菌にとっては反対に生育阻害や菌形変化に大きく作用することがある。したがって、M.

glutamicus において、上述のごとく数種のアミノ酸によって多細胞体の形成が促進されたり、システインによって伸長肥大形が形成されたりしうことはありうべきことであろう。システイン添加培地中で *M. glutamicus* を培養すると、システインは酸化されてシスチンとなり析出してくるが、システインの効果が生成されたシステインによるものとは異なることは、あらかじめシスチンを添加した培地では伸長肥大が起こらないことから明らかである。

4. 抗生物質の形態変化におよぼす影響

(1) ペニシリン

一般に、ペニシリン低濃度の存在では、菌は分裂阻害を受け、filamentous form を形成する。また L 型菌形成も良く知られるところである。

Tulasne および Vendrely⁴⁾ は *Staphylococcus* に PC を作用させ、菌形が膨大化し、その直径が 3~4 倍に達することをみ、Fleming ら⁵⁾ は PC 含有寒天にて *Proteus vulgaris* を培養し、分裂阻害の結果、菌はひも状やふくれた形など fantastic

な形をとるが、核分裂は正常であることを報告、Pulvertaft⁶⁾も PC 処理により菌体の糸状化が顕著であることを述べ、さらに上条^{7,8)}、大森⁹⁾なども数種の菌の伸長、核の正常分裂をみている。

また PC による L 型菌の形成については、Dienes¹⁰⁻¹³⁾の広範な研究があり、Tulasne も *Proteus* 菌において deoxyribonucleical granulation をおこした pleuropneumonia-like body の形成をみており、さらに *Escherichia coli*, *Bacillus anthracis*, *Salmonella*, *Proteus*, *Pasteurella* などが処理で細胞質分裂を起こさなくなるが、核分裂は正常に起こるために large body を形成することを述べている。

著者らの研究では、*M. glutamicus* においては PC の低単位 (1 mcg/ml 以下) 存在下では分裂阻害よりむしろ分離阻害がみられ、高度の多細胞体形成の結果、伸長形態をとることを知った。本菌のごとく、多細胞体形成能が強く、本質的性質とも思われる菌と、通常の単細胞体形成菌とは PC による形態変化もその性格を異にするようである。

PC により形成される多細胞伸長形態が細胞質分裂阻害の結果ではなく、分離阻害のほうが強く現われた結果であると判断される理由は、つぎのごとき実験的事実による。すなわち、基本培地中のビオチン含量を 100 γ /l に増加し、PC を添加してビオチン 1 γ /l の場合と同様に試験をしてみると、伸長多細胞体は形成されず、生育も良好である (ただし、1 日培養では 2 mcg/ml の添加で阻害される)。

ビオチン 100 γ /l 培地にクエン酸ナトリウムを加えた場合、*M. glutamicus* はビオチン 1 γ /l 培地におけると同様に伸長肥大分岐を形成する。このことは、一見伸長多細胞体の形成という点では同一と考えられるが、その形成機作の点からは PC とクエン酸ナトリウムでは異なるものと想像される。

(2) ストレプトマイシン

Stubblefield¹⁴⁾ は、SM 耐性獲得実験中、*E. coli* が異常形態を示すことを発見した。すなわち、SM 含有寒天上で分岐をもった変わった形の桿状、曲がった形、あるいは丸い形などが混合してくる。また、Pulvertaft⁶⁾ は SM 処理により菌は球状化すると述べている。

著者らの観察においてもっとも顕著な点は、SM 0.4~0.8 mcg/ml 添加で液胞と思われる構造が出

現したことである。菌形態は伸長せず、やや小型化し、楕円ないし短桿状を呈する。

(3) マイトマイシン-C

MM-C は DNA 合成阻害剤として著明であるが *E. coli* において芝ら¹⁵⁾は 0.1 mcg/ml 添加で DNA 合成が完全に阻害されることをみている。著者らは 0.2~0.8 mcg/ml 添加にていちじるしい伸長多細胞体の形成をみた。このような菌体において、はたして DNA 合成がおこなわれているか否かは興味のあるところである。形態変化と DNA 合成とがいかなる関係にあるか、MM-C は興味ある材料を提供するものと考えられる。この点はつぎの機会に検討したい。

要 約

Micrococcus glutamicus はクエン酸ナトリウムあるいはリンゴ酸ナトリウムを添加した合成培地中で伸長肥大、ときに分岐などのごとく、いちじるしい形態変化をおこなうことを知った (前報) ので、さらにこのような効果をしめす物質を探索し、つぎのごとき諸点を明らかにした。

1. クエン酸の種々の塩類のうち、ナトリウム塩に相当する効果を示すものはアンモニウム塩とカリウム塩のみである。カチオンの影響が強い。
2. 種々の有機酸のうち、シュウ酸のナトリウム塩は強い伸長肥大分岐効果をしめす。
3. アミノ酸類は一般には生育を促進するが L-シスチンは 50~100 mg/ml の濃度で生育を阻害し、50 mg/ml の添加により伸長肥大菌を形成する。
4. ペニシリン低濃度 (0.6~2.0 mcg/ml) 添加によりいちじるしい多細胞体の形成をみた。このときの菌長は数 10 μ に達する。ペニシリン添加により形成される多細胞体は、ビオチンを 100 γ /l 添加することにより形成されなくなる。このことより、ペニシリンは細胞質分裂阻害より、むしろ細胞分離阻害と考えられる。
5. ストレプトマイシンは菌形態を小型化せしめる。0.4~0.8 mcg/ml 添加により、菌体中にかなり大きい液胞様構造が出現する。
6. マイトマイシン C は、0.2~0.8 mcg/ml 添加によりいちじるしい多細胞体の形成をみ、菌全長はときに 10 μ を越えることもある。このような菌

は、概して末端細胞は膨大化し、いわゆるこん棒状を呈するが、その反対に、末端が縮小したものもよく観察された。

終始ご指導、ご助言をいただいた東大教授湯浅明博士、東大教授北原覚雄博士に深く感謝いたします。また、種々実験にご協力いただいた当所員垣下祐子氏に感謝いたします。

文 献

- 1) 板垣史郎・木幡 守・木下祝郎, 植維 **74**: 452 (1961). 2) 板垣史郎・木下祝郎, 同 **72**: 51 (1959). 3) Dienes, L., and Zamecnik, P. C., J. Bact. **64**: 770 (1952). 4) Tulasne, R., and Ventrely., Nature **161**: 316 (1948). 5) Fleming, A., Voureka, A., Kramer, I. R. H., and Huges, W. H., J. Gen. Microbiol. **4**: 257 (1950). 6) Pulvertaft, R. J., J. Path. Bact. **64**: 75 (1952). 7) 上条清明, 細菌誌 **8**: 747, 795 (1953). 8) —, 同 **9**: 128, 193, 253, 305 (1954). 9) 大森史郎, 米子医雑 **7**: 503 (1956). 10) Dienes, L., J. Bact. **56**: 445 (1948). 11) —, Proc. Soc. Exp. Biol. N. Y., **68**: 589 (1948). 12) —, ibid. **75**: 412 (1950). 13) —, ibid. **83**: 579 (1953). 14) Tulasne, S., Nature **164**: 876 (1949). 15) Stubblefield, E., J. Bact. **54**: 81 (1947). 16) Shiba, S., Terawaki, A., Taguchi, T., and Kawamata, J., Nature **183**: 1056 (1959).

Summary

It was reported in a previous paper that the elongated, enlarged and even branched form of *Micrococcus glutamicus* was observed in the synthetic medium containing Na-citrate or Na-malate.

As the result of the investigations on substances having the effect like that of Na-citrate or Na-malate, following informations were obtained obviously.

1. Among various salts of citrate, ammonium and potassium salts showed the similar effect to that of Na-salt on the morphological change.

2. Na-oxalate also has a strong elongating and branching effect.

3. Generally, amino acids promote the cell growth of *M. glutamicus*. The large quantities of L-alanine and L-cystein, however, inhibit its growth. The elongated and enlarged cells were formed by the addition of 50 mg./ml. of L-cystein.

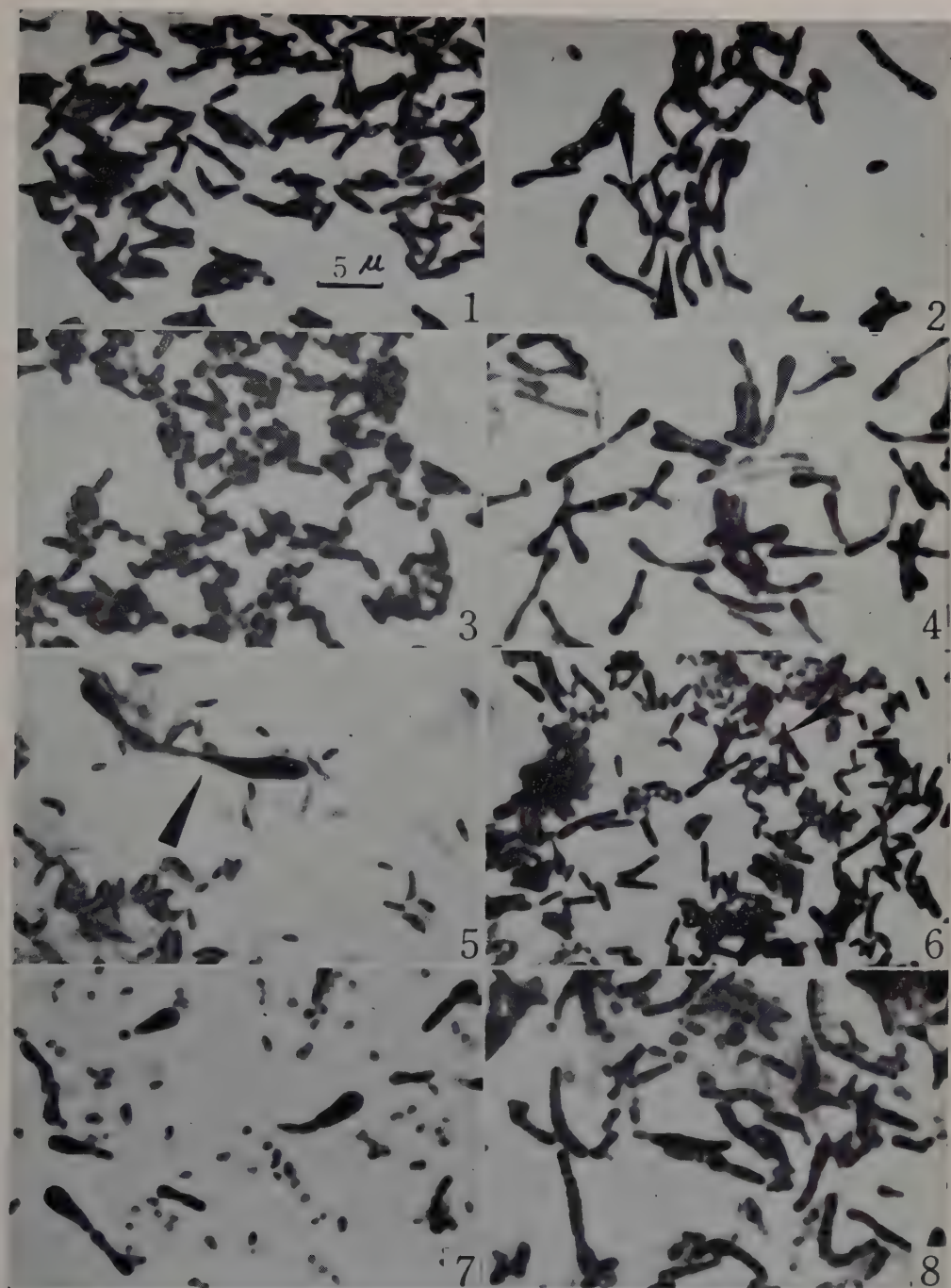
4. High multicellular and elongated bodies were formed in a medium containing the low level of penicillin. Such multicellular bodies, however, were never formed in the penicillin media if the biotin content was increased to 100 γ /l. From this fact, it seems that penicillin strongly inhibits the separation of the daughter cells rather than the cytoplasmic division.

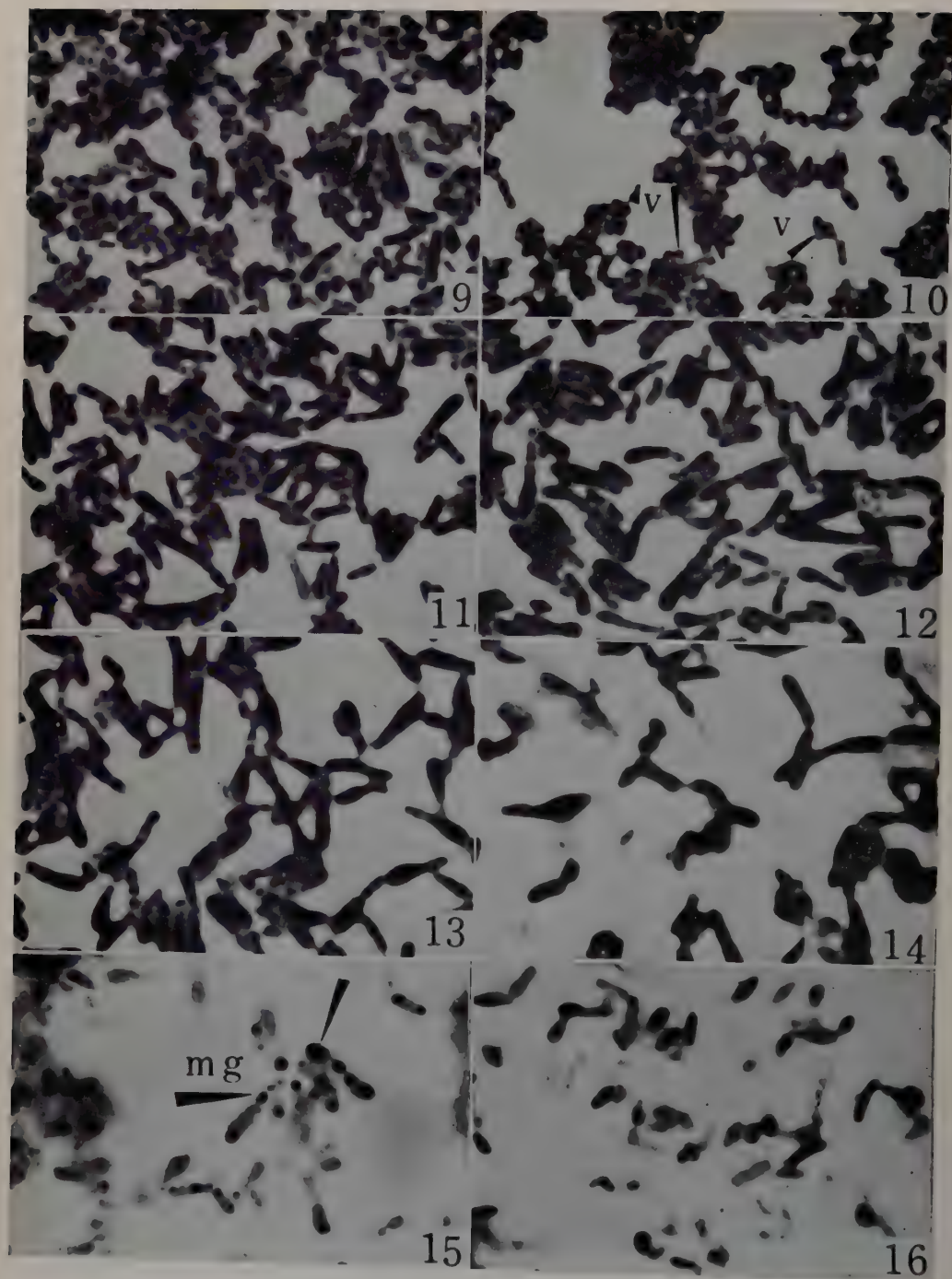
5. The multicellular bodies never formed in a medium containing streptomycin. But a vacuole-like structure in each cell was formed in the concentration of 0.4–0.8 γ /ml. of streptomycin.

6. Remarkable multicellular bodies were formed in the concentration of 0.2–0.8 γ /ml. of mitomycin-C. In these cases, the length of cell is 10 μ or over occassionally. One or both ends of these cells were swollen and become so-called club-shape.

写真説明

1. クエン酸アンモニウム 4%, 2日培養. やや長い bifid bacteria 型を呈する.
2. クエン酸カリウム 6%, 2日培養. 菌体はかなり伸長し, 分岐も認められる.
3. クエン酸銅 0.05 飽和, 2日培養. 菌長は短かいわりに多細胞体である.
4. シュウ酸ナトリウム 2%, 2日培養. 良く伸長し, 末端は膨大, こん棒状を呈する.
5. シュウ酸ナトリウム 4%, 2日培養. 全般に菌体は伸長しないが, ときに異常に伸長肥大した菌が混在する.
6. アゼライン酸ナトリウム 8%, 2日培養. 菌体の伸長度はあまり大ではないが, 分岐も認められる.
7. シスチン 50 mg/ml 2日培養. 菌生育は不良で, 大部分の菌体は小型であるが, 末端いちじろしく膨大した伸長肥大菌が混在する.
8. ペニシリン 7.8 mcg/ml 2日培養. いちじろしい伸長多細胞体を形成, しかし菌形は不育化している.
9. ペニシリン 4.0 mcg/ml, 2日培養. 大部分の菌は2細胞の状態であり, 混在する多細胞体もその程度を減じている.
10. ストレプトマイシン 0.4 mcg/ml 1日培養. 菌形は楕円形ないし短桿状で, 液胞様構造(v)を形成している.
- 11—16. マイトマイシン添加.
12. 0.6 mcg/ml, 2日培養.
13. 0.8 mcg/ml, 2日培養. 0.4~0.8 mcg/ml まで, 漸次, 多細胞体の程度を増している.
14. 1.0 mcg/ml, 2日培養. やや多細胞体の程度を減ずる.
15. 2.0 mcg/ml, 3日培養. いちじろしく大きい metachromatic granule (mg) を多数形成している.
16. 4.0 mcg/ml, 2日培養. 生育は完全に阻害され, 小型菌が散在する.





ナンジャモンジャゴケの知見補遺*

井 上 浩**

Hiroshi INOUE**: Supplements to the Knowledges
on *Takakia lepidozioides* Hatt. et Inoue*

1961 年 6 月 16 日受付

ナンジャモンジャゴケ (*Takakia lepidozioides* Hatt. et Inoue) は記載されていらい¹⁾, その特異的な形態から分類学上の位置が問題となっている。服部・水谷²⁾ は本種が Carobryales に近いものであるとし, さらに辰野³⁾ は核学的研究から本種の核型が $n=4=V(H)+V+J+J(h)$ であり, 蘚苔類中で最少の染色体数をもつものとし, これを基本型として蘚苔類の核型の進化を論じた⁴⁾。

本種は Takakiales として独立目とみなされるが, 記載当時はその生殖器官も発見できず, ステリルのままで発表された。その後, 服部・水谷²⁾ および服部⁵⁾ は造卵器を発見, 記載した。葉序については, 服部・井上¹⁾ は“一言にしていえば不規則というほかない”としたが, 服部・水谷^{2,6)} は基本的には三列に生じるとした。

現在までの形態学的な知見は, ミクロトーム切片によってえられたものではなく, 外部観察かまたはかんたんな解剖手段によってえられたものである。本報においては, 1958 年 8 月に長野県白馬岳において採集した資料, および 1959 年 8 月富山県立山において採集した資料を用いて, ミクロトーム連続切片を作成してえられた知見を報告する。材料はすべてクロム酢酸液で 12 時間固定, パラフィン法で 5μ の厚さの切片とし, ヘマトキシリン (ハイデンハイン法) によって染色した。

* 東京教育大学理学部植物学教室 および 服部植物研究所業績。本報の一部は 1961 年 4 月植物学会関東支部大会において発表した。

** Botanical Institute, Faculty of Science, Tokyo University of Education, Tokyo, Japan.
東京教育大学理学部植物学教室

観 察 結 果

1. 茎頂部の構造

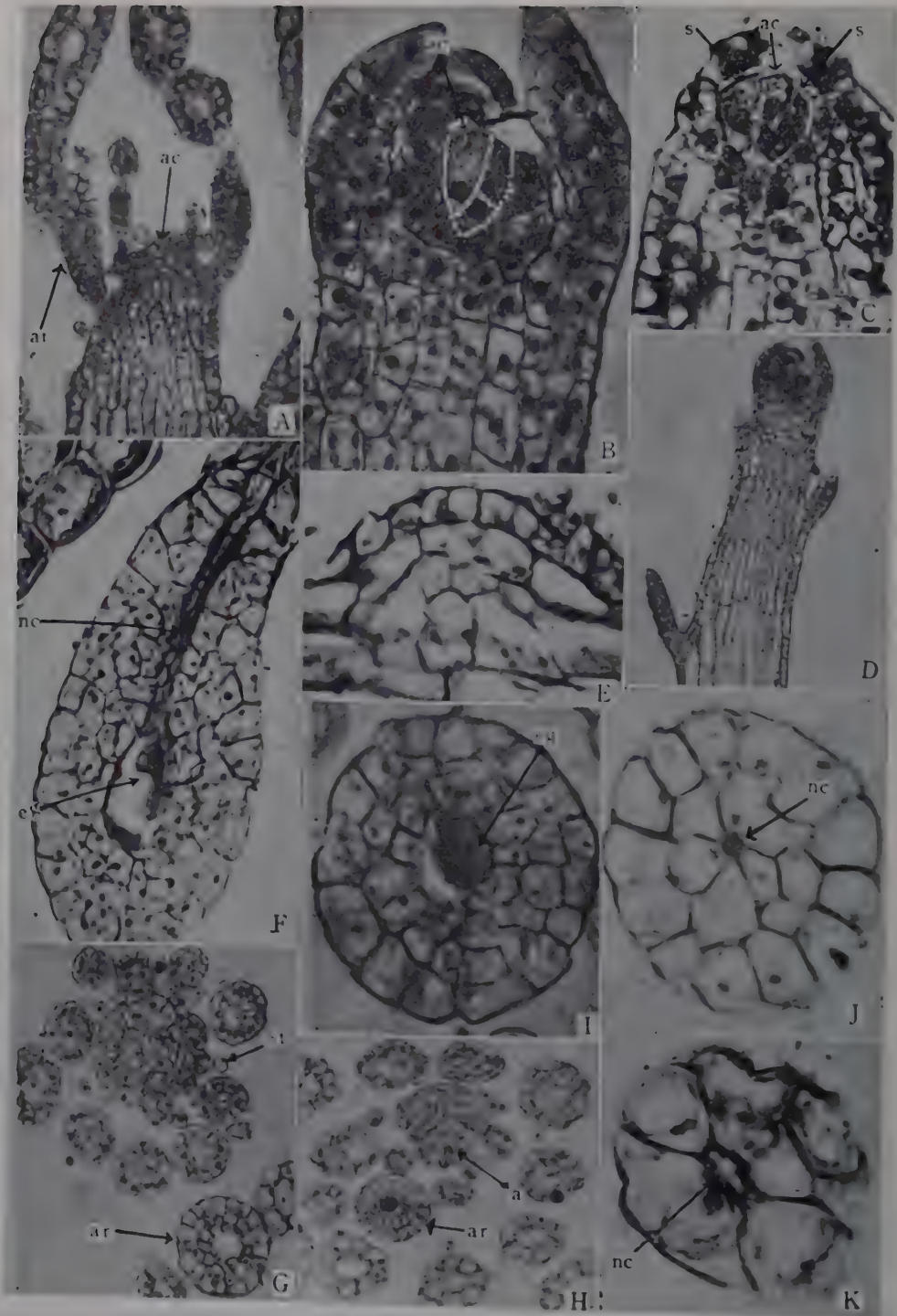
茎頂部の横断面 (第 2 図 A) では, 三角形をした頂端細胞がみられる。頂端細胞は三方向に分裂面をもっており, この各分裂面に接して比較的大きな細胞がある。茎頂部の縦断面 (第 1 図 B, C) では, 頂端細胞はピラミッド状で, これに接する細胞は頂端細胞とほぼ同じ大きさである。頂端細胞の分裂によって生じる 3 個の細胞は, いずれもほぼ同大で差はみられない。

頂端細胞の分裂によって形成された 3 個の細胞は, まず頂端細胞の分裂面にほぼ直角に第一回の分裂をする (第 1 図 B)。ここで形成された 2 細胞のうち内部に位置する細胞は, 茎の内部の細胞に分化していくものであり, 外部に位置した細胞は, 茎の表皮およびこれにつづく 2~3 層の細胞, および葉の細胞に分化するものである。なお, 頂端細胞付近の数個の細胞の内部には葉緑体がみられない。

2. 葉序の問題

本種の最大の特徴の一つは, その葉序が不規則である点であったが, 服部・水谷²⁾ および服部⁵⁾ は原則的には一対の葉が三列に配列しているとしている。

茎頂部の構造からも明らかなごとく, 葉は頂端細胞の三分裂面のおのおのから一葉ずつが形成され, 前述のごとく, 頂端細胞の分裂によって形成された細胞 (第 1 図 B, C の s) の第一分裂によって形成された外側の細胞から発生している。第 2 図 A は頂端細胞付近での茎頂部の切片を示すが, 1~9 は各葉が茎に着生する順序を示すものである。第 2 図 B~F は, A の 1~9 の各葉が茎に着生する順を連続切



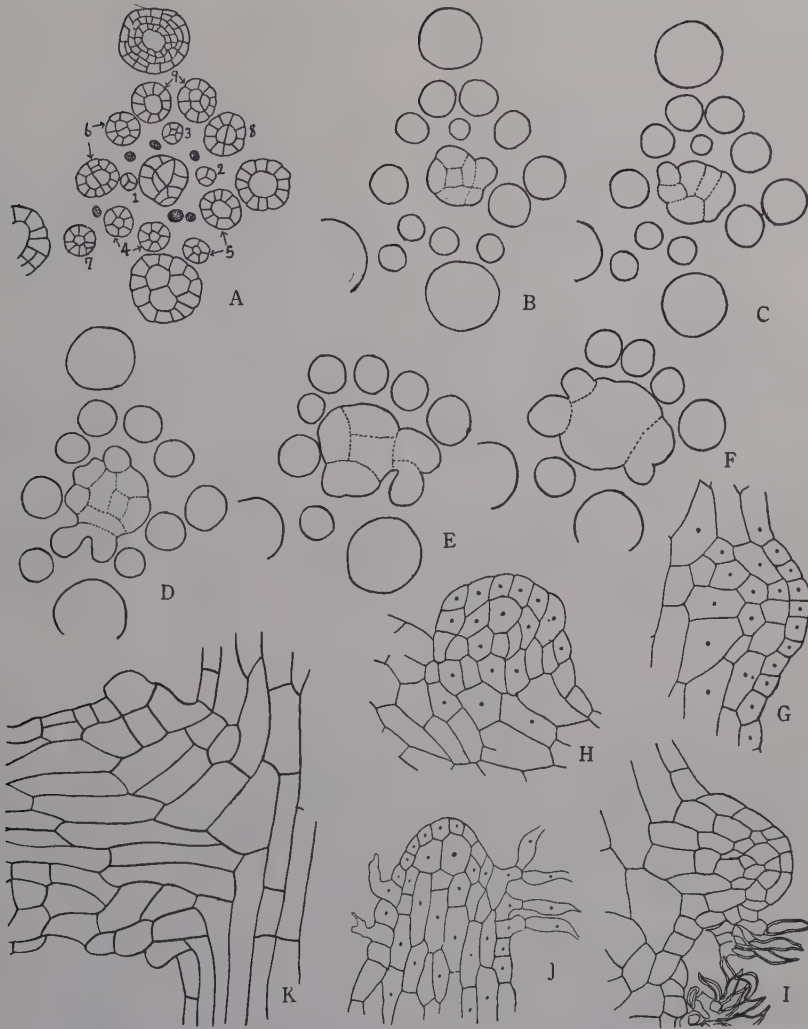


Fig. 2. Microtome sections of *Takakia lepidozioides*. A-F, successive sections of shoot apex with ca. 10 μ interval, \times 140. Numbers (1-9) of fig. A indicate leaf order from apical cell. G-K, development of flagellum, \times 300.

Fig. 1. Photographs of microtome sections of *Takakia lepidozioides*. A-D, longitudinal sections of shoot apex; E, longitudinal section of flagellum primordium; F, longitudinal section of archegonium; G-H, cross sections of shoot apex; I, cross section of venter of archegonium; J, ditto of lower part of neck. K, ditto of upper part of neck. ar: archegonium. ac: apical cell. s: cells sided to apical cell. nc: neck canal. eg: egg cell. st: stylus. a: shoot apex. A, \times ca. 200; B, \times ca. 400; C, \times ca. 300; D, \times ca. 80; E, \times ca. 250; F, \times ca. 900; G-H, \times ca. 400; I, \times ca. 1200; J-K, \times ca. 1300.

片によって示したものである。これらの諸図に見られるように、茎の頂端部付近では、葉は頂端細胞の三分裂面にしたがって配列している。しかし、茎の頂端部から離れるにしたがって、頂端細胞の分裂面に対する位置からねじれを生じている。これは、頂端細胞の分裂が逐次的におこることによるものであらうと考えられる。このような位置のねじれによって、各葉はらせん状に茎に配列するようになっている。

葉の原基は最初はかならず単一で、これからは一葉を形成するのが原則的である。しかし、この原基が発達途中で二分して二葉を形成する場合がみられる(第2図Aの4, 5, 6, 9の各葉のごとく)。このような葉においては、二葉が対をなしているが、対になった葉の基部はかならずゆ合している。

3. 造卵器の構造

本種は最初ステリルの状態で記載されたが、服部・水谷²⁾は Queen Charlotte Is. 産の資料で造卵器を記載し、その後、服部ら³⁾は白馬岳産の資料でも造卵器を記載した。立山産の資料中にも多数の造卵器が発見された⁴⁾が、現在までには造精器は発見できていない。

造卵器の発生は茎の頂端細胞の近くでおこなわれているが、頂端細胞そのものはまったく関係していない。第1図A, G, Hおよび第2図Aからうかがえるように、造卵器の位置は一葉が位置すべき位置にはば一致している。

造卵器のあしは長く、造卵器の全長の約1/4を占め、4～6細胞の厚さである。腹部は2～3細胞層の壁をもち、横断面ではほぼ円形に近い(第1図I)。卵細胞は、若い造卵器では明りょうであるが、古い造卵器ではすでに消失しているものが多い(第1図F)。多数の造卵器を観察したが、卵細胞はいずれも分裂をはじめることなしに消失している。このことは、受精がおこなわれなかったことを明らかに示している。

造卵器は腹部からくびに移るところで、多くの場合やや急にせばまっている。壁の厚さは2～3細胞層から1細胞層となる(第1図F, J, K)。くびの部分は10～15細胞の長さで、たてに6列に並んでいる(第1図K)。けいこう細胞の数はわからなかったが、けいこう部は比較的せまく、けい細胞の厚さの約2/3以下である。茎の頂部から離れたところにあ

る造卵器では、ほとんどの場合、けい細胞の部分⁵⁾が褐色に変色して消失しかけている。茎の頂部付近の造卵器は淡緑色で、葉緑体が各細胞内にみられる。しかし、この葉緑体は葉の細胞内のものよりずっと小形で、量も少ない。

4. 枝の発生

茎の基部付近からは、通常数本のべん枝を出している。これは腐植土内をはっているが、その先端部から新しい茎葉体が発達することがある。一種の枝と考えてよいと思われる。植物体全体としては仮軸分枝となっている。

べん枝の発生は、茎基部の表皮細胞のすぐ下の1～2層の細胞(皮層にあたる)からおこっている(第1図E, 第2図G)。この部分の細胞が分裂組織になると、つづいて表皮細胞も分裂をはじめ小形となる(第2図G, H)。内部の分裂組織の細胞は次第に小形の細胞となりながら、表皮細胞とともに表面に突出してくる。このころ、すでに共生菌糸が付着していることが多い(第2図I)。この菌糸は表皮細胞にはいつているが、それ以内には進入していない。表皮細胞は菌の共生によって変形されている。

茎の中部から発生してくる枝は、上記のべん枝の場合とほぼ同じような過程をたどるが、菌におかされることはない。発生をはじめた枝またはべん枝はともにその頂端部一層の細胞が小形で、頂端細胞はみられない(第2図J)。おそらく、頂端部の細胞全体が分裂組織として働いているものと考えられる。しかし、ある程度に伸長した枝やべん枝では、茎の頂端細胞と同様な頂端細胞が形成されている。

いずれの場合でも、枝およびべん枝の基部は、茎の表皮細胞から内部へ1～2細胞層しかはいっていない(第2図K)。このことと関連して、枝などでは容易に茎から脱落しやすい性質がみられる。

考 察

ミクロトーム切片による上述の観察結果は、すでに服部らによって観察されたものとだいたい一致している。本論文はその確実な論拠を与えるものである。

服部・水谷²⁾は、Queen Charlotte Is. 産の資料で造卵器のくびは4列の細胞よりなると記載しているが、上述の観察では白馬岳産および立山産いずれの資料でも6列になっていた。造卵器の構造は、服部ら^{2, 5, 6)}の論議のごとく、ごく原始的と考えられ

る。造卵器は、上述したように明らかに葉と同一の起原をもち、葉の細胞と同じく少量ではあるが、造卵器の細胞内にも葉緑体がみられることは、造卵器の葉よりの分化がごく少ないことの一論拠となると考えられる。造卵器の構造は、苔類の造卵器よりも蘚類の造卵器に類似した点が多い。

頂端細胞の形および分裂は、Carobryales や Jungermanniales acrogynae などの苔類および蘚類一般にみられるものと大差ない。頂端細胞の三分裂面がほぼ同じ大きさである点（このことは植物体に背腹性がないことの原因となっている）は、蘚類に近い形態となっている。茎頂部では、葉が頂端細胞の各分裂面に一葉ずつ形成され、らせん状に配列することも蘚類に近い葉序とみられる。

摘 要

ナンジャモンジャゴケのミクロトーム切片を作成して研究した結果、次のことが明らかとなった。

1. 茎の生長点では、1個の大形な頂端細胞とこ

れに三面で接する3個の大形細胞がみられる。頂端細胞の分裂によって形成された3個の細胞は、ほぼ同じ大きさで背腹性を示さない。

2. 茎の生長点付近では、葉は頂端細胞の各分裂面に対し一葉ずつ形成され、各葉はらせん状に配列する。茎頂部をはなれるにつれ、各葉は位置にねじれを生じている。

3. 造卵器の腹部は2～3細胞層の壁をもち、けい細胞は6列に配列する。造卵器は葉と同じ起原をもち、葉よりの分化程度は少ない。

4. 分枝は、茎の表皮細胞から1～2層内部の細胞が分裂組織となって形成される。

5. ナンジャモンジャゴケの造卵器および生長点の構造は蘚類との密接な関係を示すことが多い。

稿をすすめるにあたり、ご指導をいただいた伊藤洋教授に感謝する。また、服部新佐博士には資料採集にあたりご援助をいただき、原稿に対し種々ご批判をいただいた。記して深く感謝の意を表する。

文 献

- 1) 服部新佐・井上 浩, 服部植物研究所報告 19:133 (1958). 2) ———・水谷正美, 同 20:295 (1958). 3) 辰野誠次, 同 20:119 (1958). 4) ———, Cytologia 24:138 (1959). 5) Hattori, S. Mizutani, M., Inoue, H., Journ. Jap. Bot. 33:321 (1958). 6) 服部新佐, 蘚苔地衣雑報 1(17):1(1958). 7) 井上 浩, 蘚苔地衣雑報 2(2):14 (1960).

Summary

Takakia lepidozoides Hatt. et Inoue was studied by microtome sections, and the followings were revealed:

1. The apical cell at shoot apex is tetrahedral and has three cutting faces. Three cells cut off from the apical cell are all of the equal size (Fig. 1, A-C).

2. At shoot apex the leaves are in three vertical rows corresponding to the three cutting faces of the apical cell of stem. Later, however, the leaves displace and lose their arrangement in three ranks. The leaf primordia often divide and a pair of leaves develops (Fig. 2 A-F).

3. The venter of archegonium has the wall of 2-3 cells thick, and neck cells arrange in 6 vertical rows. The archegonium is of the same origin with a leaf, and it has a few chloroplasts in the cells (Fig. 1, F-K, and Fig. 2, A).

4. Branches are originated from the cortical cells, which become meristematic (Fig. 1, E, and Fig. 2, G-K).

5. The structures of shoot apex and of archegonium are largely similar to those of Musci.

メタセコイアの樹皮, 特に巨大コルク細胞について

肥田美知子*

Michiko HIDA*: Studies on the Bark of *Metasequoia glyptostroboides*
with Special Reference to Giant Cork Cells

1961 年 7 月 15 日受付

樹木における樹皮の形態は植物の種類によって特異的で、しばしば種の判別点にもなる。メタセコイアでは新条は緑色で、一年後には茎の表面は細く縦裂した樹皮でおおわれ、その下には緑色の周皮がある。その後、樹皮は輪状に剝離し、枝の表面には褐色の粒状物が付着している。この型の剝離は2—4年を経た枝にだけみられるものである。したがって若い木では下枝もこの剝離をするが、年をへた木では上枝にだけみられる。さらに古い枝では表面はまったく平滑になり、枝が太くなるにつれてところどころに縦の裂目ができる。幹では表面は縦裂した樹皮につつまれ、漸次、表面からはがれ落ちる。

このような樹皮の形成段階を組織学的にしらべた結果、粒状物は師部に由来する大型のコルク細胞であることがわかったので、ここに報告する。

材料および方法

大阪市立大学および大阪女子大学のメタセコイアからいろいろの若さの枝をとり、手切切片を作り樹皮の構造を比較した。また、各時期の樹皮を硝酸と過マンガン酸カリで解離したもの、ならびに輪状にはげる樹皮の内側に付着している粒状物について、その構成要素を観察し、大きさを測定した。コルク細胞の大きさは遊離細胞の面積であらわした。なおコルク形成層の出現場所を知るために、切枝を約19時間テトラゾリウムの 1 mg/1 ml 水溶液につけ、

切片を作ってしらべたが、この方法は第一期のコルク形成層の検出には有効であったが、他の時期においては思わしい結果が得られなかった。

観 察

1. 樹皮の形成

短枝をつける新条は緑色で、時には赤味を帯びている。横断面は丸味を帯びた方形で四隅に葉が沿下して稜を作っている。

横断面において1層の表皮の下に1—2層の厚膜細胞よりなる下表皮が点々として存在する。皮層のうち下表皮から2層目あたりから2—4層の細胞は特に大きく切線方向に長くのび、葉緑体をほとんど含んでいない。のちに、この組織の内側にコルク形成層ができる。皮層の4か所、すなわち、方形の各辺の中央部に樹脂道がある。師部の繊維はまだよく発達しておらず、師部の周辺部にわずかに存在しているにすぎない。

新条は次第に表面が褐色になり、Fig. 6Aのように樹皮は縦裂し、一部はげ落ちる。それ以後の枝の表面は緑褐色でなめらかである。樹皮の落ちる前の横断面は Fig. 2 にみるように皮層の中部、すなわち大きな細胞の内側にコルク形成層ができ、外部にコルク組織を、内部にコルク皮層を形成している。テトラゾリウムにつけた枝ではコルク形成層はうすく染った。コルク組織より外側の皮層細胞はやや厚膜化し、コルク化している。縦にはげ落ちるのは一部のコルク組織と、それより外のコルク化した皮層、下表皮および表皮で、剝離後の枝の表面は2—3層

* Biological Laboratory of Osaka Women's University, Osaka, Japan. 大阪女子大学生物学教室

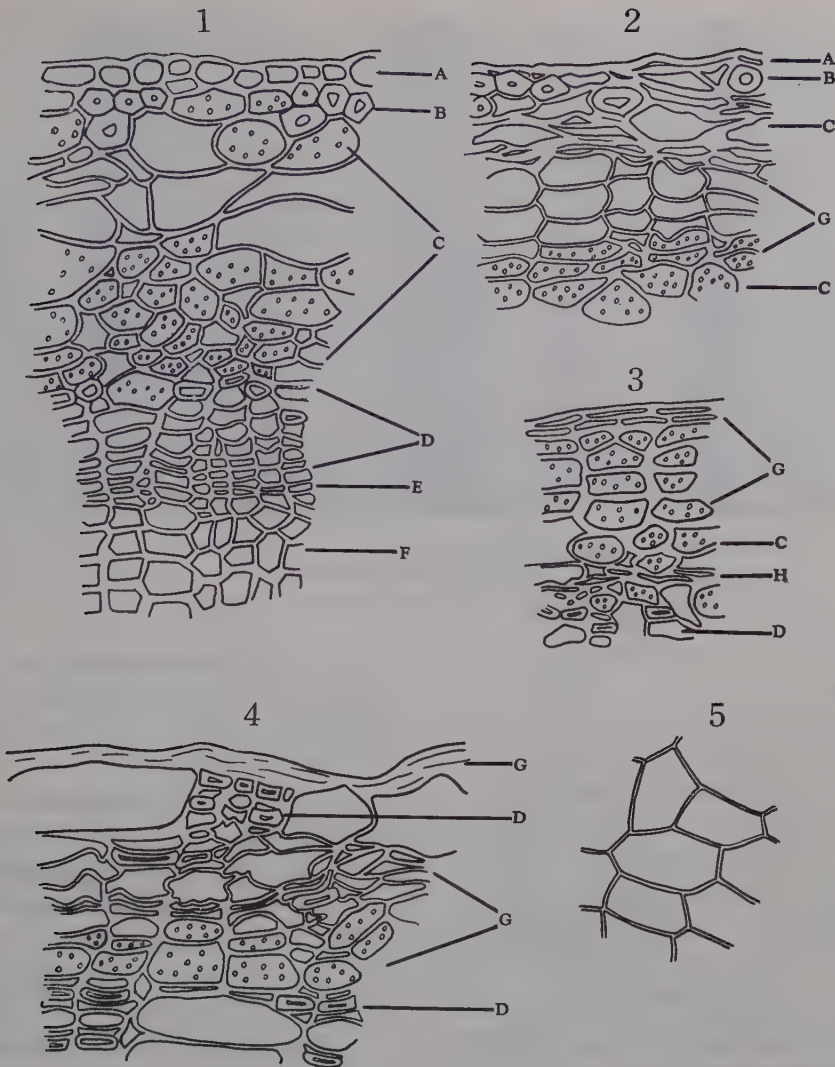


Fig. 1—4. Transections of twigs of *Metasequoia* of various ages ($\times 600$).

1, a young greenish twig; 2, upper part of a twig, one year old; 3, basal part of a twig, one year old; 4, a twig, 2—4 year old.
A, epidermis; B, hypodermis; C, cortex; D, phloem; E, cambium; F, xylem; G, periderm; H, initial of next periderm.

Fig. 5. Surface view of the young cork tissue ($\times 600$).

の Cork 組織と 2—3 層の Cork 皮層とよりなる周皮でおおわれている。この時期の Cork 細胞は表面観は五、ないし六角形で (Fig. 5), 横断面では半径方向にやや平たい矩形をして半径方向に正しく並んでいる。解離した Cork 細胞について測定した細胞の大きさは $3,575 \mu^2$ くらいであった。樹皮がはげ落ちた後は内鞘のあたりにあらたに形成層が生じ新

らしく周皮が作られる (Fig. 3)。このころには師部はよく分化し、ニオイヒバ (*Thuja occidentalis* L.) などにみられるように、師部繊維、師管、柔細胞が半径方向に並んでいる¹⁾。

2—4 年枝になると、樹皮は縦裂でなく輪状ないし方形に大きくはげ (Fig. 6B), その内部に黒褐色の肉眼でもわかる粒状物が枝の表面をおおっている。

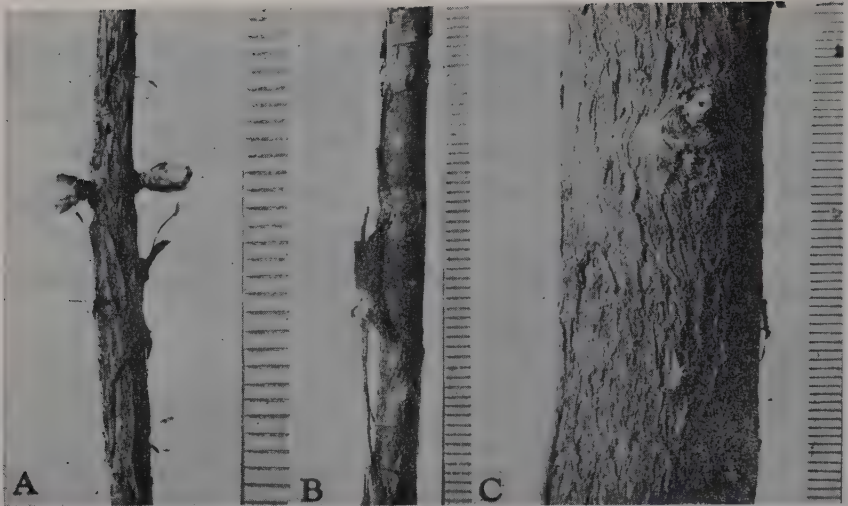


Fig. 6. The twigs of *Metasequoia*.

A, a twig, one year old (bark cracks lengthwise); B, a twig, 2–4 year old (bark sloughs away in rings); C, a twig over 4 years old.

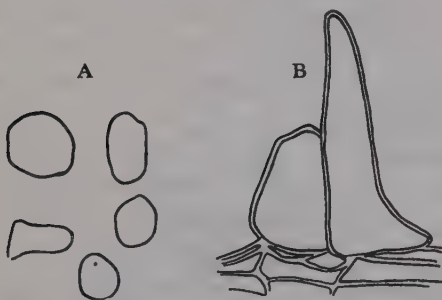


Fig. 7. Giant cork cells.

A, free giant cork cells ($\times 120$);
B, those attached to the surface of the periderm ($\times 600$).

剝離した部分は2年枝では周皮、皮層の残部および師部の一部で、3–4年枝ではコルク形成層がさらに内部、すなわち師部のなかにできるために、周皮および師部の一部よりなっている。枝の表面につく粒状物は大きなコルク細胞で (Fig. 7), そこにみられる3糸状物は師部の繊維細胞である。

さらに年をへた枝では表面が比較的平滑になり周皮におおわれているが、枝の生長につれて次第に縦裂し (Fig. 6C), 幹は縦裂した樹皮で包まれている。すなわち、縦裂した樹皮はすぐはげ落ちず幹を包み、表面から徐々にはがれる。この樹皮を解離してみる

と、コルク細胞と師部の繊維細胞からなっていた。しかし、2–4年枝の表面にみられるような大型コルク細胞は見あたらなかった。

2. 大型コルク細胞

2–4年枝の輪状にはげた樹皮の内側に付着している粒状物は Fig. 7 の A のようで、球形または楕円形の褐色の遊離細胞で、細胞膜は比較的薄く、コルク化している。この細胞約 1000 個について面積を測定して作った変異曲線が Fig. 8 で大きさの範囲は $1,430-44,330 \mu^2$ で、そのうち $10,010 \mu^2$ のものがもっとも多く、非常に大きなコルク細胞であることがわかった。この細胞がどこから由来するものかを知るために、各期の樹皮を解離して細胞の大きさを測定したが、大きさにおいては新条の皮層の細胞がややこれに近いだけで(大きなもので $10,010-11,440 \mu^2$), 他の細胞はいずれもはるかにこのコルク細胞より小さく、樹皮を形成しているコルク細胞は $3,500 \mu^2$ くらいにすぎない。したがって、最初は大細胞が2年枝にみられることから皮層細胞のコルク化したものと考えたが、その後、3, 4年枝にも存在すること、またこのころの剝離してくる樹皮はコルク細胞および師部繊維からなっていること、さらに、枝の周皮の表面になかば円化した細胞が付着していること (Fig. 7, B) からしてコルク組織の内部、すなわち、師部の細胞が枝の急激な生長に

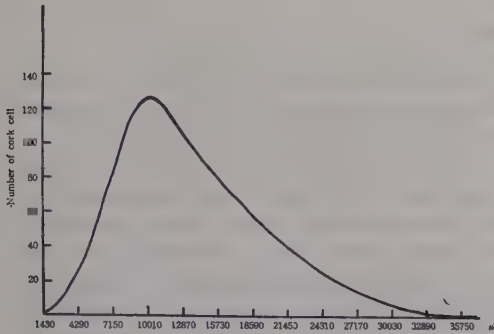


Fig. 8. Variation curve of giant cork cells.

つれていちじるしく発達し円化，遊離したものであることがわかった。このことは粒状物が若い木では下枝にもみられるが，年をへたものでは上部の发育のさかんな枝にだけしかみられないことをも説明できる。

考 察

樹木において剝離してくる樹皮の形はいろいろで^{2,3,4,5,6}，樹木の一特徴とされているが，同一樹木でも年令や枝と幹によって違った形を示す場合がある。メタセコイアでは観察の項に記したように，若い1—2年枝では縦裂し，各裂片ははそくはげ落ちる。幹も同様に縦裂するが若い枝より幅広く縦裂し，スギ (*Cryptomeria japonica*) などで見られるように年々形成される樹皮は堆積し，その表面からごくわずかずつはがれていく⁷。1年枝の樹皮は表皮，下表皮，皮層細胞からなり，この組成中の下表皮の繊維細胞で長くつながったようになり，幹の縦裂片では師部繊維が下表皮にかわっている。

2—4年枝では縦裂でなく輪状剝離をする。これはサクラ，ブドウなどにみられる型であるが，松柏類ではヒノキ科のあるものにみられる⁴。この時期の樹皮はコルク細胞とごくわずかの師部繊維からなり，その内側に師部繊維をまじえた遊離したコルク

細胞からなっている粒状物が存在する。このようなことは2—4年枝だけにみられることで，相当年をへた木では，下枝にはこの剝離型はみられず，枝の表面は比較的平滑であるが，上部の枝では輪状剝離を示す。したがって，このような剝離法はメタセコイアでは非常に生長のさかんな時期に起こるものと思われる。

このとき，枝に付着している粒状物は大型コルク細胞で平均 $14,586 \mu^2$ あり，肉眼でも粒状と判断することができる。この由来については上記の通り，生長の非常にさかんな時期に周皮の内部の師部の細胞が特殊に肥大し円化したものと考えられる。このように師部を構成する細胞が特殊に膨張することはすでに島倉によって指摘されたことで⁸，スギ科ではコウヤマキ (*Sciadopitys verticillata*) やコウヨウザン (*Cunninghamia lanceolata*) などにもみられる現象であるが，いずれの場合にもメタセコイアのように円化し，遊離することはみられない。セコイアメスギ (*Sequoia sempervirens*) の幹では固着した周皮が幹の表面をおおい，その内部に綿状のものがある。これはコルク細胞と繊維細胞からなっているが，この場合にもメタセコイアにみるようなコルク細胞の円化および遊離はみられず，もとの組織の形態を残している。

メタセコイアの周皮の細胞は最初の年はマツ属 (*Pinus*)，カラマツ属 (*Larix*)，イチチョウ科 (*Ginkgoaceae*) のものと同じように皮層の中部に生じ，第二期コルク形成層は師部の外層，すなわち内鞘の部分に，第三期以後は師部の内部に生じる。ヒノキ科 (*Cupressaceae*) のビャクシン属 (*Juniperus*) などでは第一期コルク形成層が皮層の内部に生じることが知られている²⁻⁴。

終わりにのぞみ，ご懇切なご指導を賜わった大阪市立大学理学部の三木茂博士に厚くお礼申し上げます。

文 献

- 1) 島倉巳三郎，植維 50 : 206 (1936)。
- 2) Eames, A. J., and MacDaniels, L. H., An Introduction to Plant Anatomy, pp. 248—276, N. Y. (1947)。
- 3) 猪野俊平，植物組織学 pp. 381—386, 東京 (1954)。
- 4) Esau, K., Plant Anatomy pp. 324—337, N. Y. (1953)。
- 5) 小倉 謙，植物形態学 pp. 220—224, 東京 (1935)。
- 6) Pfeiffer, H., Die pflanzlichen Trennungsgewebe in K., Linsbauer, Handbuch der Pflanzenanatomie 5, Berlin (1928)。
- 7) 三好東一・島倉巳三郎，日本林学会誌 17 : 877 (1936)。
- 8) 島倉巳三郎，植維 50 : 318 (1936)。

Summary

1. The bark of the twigs of *Metasequoia* cracks lengthwise when it is about one year old, while the bark of two to four year old twigs splits in rings. The thick periderm which covers the trunk splits vertically and gradually sloughs away from the surface.

2. The surface of the bark of 2–4 year old twigs which peels off in rings is covered with numerous brown particles. These particles are giant cork cells. They are spherical or ellipsoidal in shape and averaging about $14,600 \mu^2$ in their surface area. Those cells originate from the expanded parenchyma of the bast, taking a spherical or ellipsoidal shape and then separate from the tissue. The phenomenon is characteristic of the bark of *Metasequoia*.

3. The first cork cambium initiates in the middle of the cortex and the 2nd periderm arises from the pericycle. The periderm of twigs of more than three years old originates in the phloem.

Chlamydomonas moewusii var. *rotunda* の暗所 接合におよぼす照明培養上清液の影響*

坪 由 宏**

Yoshihiro Tsubo*: Effect of the Supernatant of Illuminated Culture
on Dark Mating in *Chlamydomonas moewusii* var. *rotunda**

1951 年 7 月 19 日 受付

藻類の有性生殖には栄養の欠亡と同時に光が必要であることが、Klebs¹⁾ いろいろしばしば報告されており、単細胞緑藻クラミドモナスについても同様のことが数多くの種について知られている²⁻⁴⁾。ところが、この、藻類の配偶子の接合に対する光の必要度は、各研究者がそれぞれ用いた種によりたがいに異なったものがある。Lewin⁵⁾ の報告している *Ch. moewusii* では、その両接合型とも接合のために光を必要とし、Förster & Wiese^{6,7)} によれば、*Ch. eugametos* では雌株は暗所でも接合する能力を有し、雄株は光を必要とするという。一方、Sager & Granick⁸⁾ によれば *Ch. reinhardi* は前記の2種と異なり、暗所でも生長しうが、この種では酢酸ソーダの存在下に低濃度の窒素化合物をふくむ培地に生育した細胞は、光をあてなくてもある程度接合がおこるという。

筆者が分離し、これまでその生殖過程について研究を続けている *Ch. moewusii* var. *rotunda* でも増殖中の藻を窒素源を加えない液体培地に移し、光を与えた場合に、さかんな有性生殖が見られることはすでに報告されたり、しかし今回、光のもとであらかじめ寒天培地に十分に生長した藻では——この種は暗所では生育しないものであるが——暗所でもすでに接合能力をもつこと、光は細胞の接合能力を

高める働きのあること、さらに、他の種について報告されているものと異なり、光のもとで高い接合能力をもつようになった培養の上清液は暗細胞の接合能力を高めることが新しく見出された。本文ではこの藻の接合に対するこれら光の作用について調べられた予備的な実験の報告をおこなう。

材 料 と 方 法

Chlamydomonas moewusii Gerloff var. *rotunda* Tsubo^{4,9)} は筆者により分離されたもので、雌雄異株、雌雄同型である。この藻は白色、温白色蛍光灯混合による連続照明下（およそ 2,000 lux）で無機塩培地* (M-N とする) に増殖する。実験中の培養温度はおよそ 25° である。

配偶子を得る方法：(+)、(-) 各株の藻懸濁液をそれぞれ別々に無機塩培地プレート表面にひろげ 10~15日間光をあて、十分に藻を生長させる。ついで窒素源を加えない液体培地 (M-N) に藻を移し、暗所に 15~20 時間保つ。その後光を与えれば接合能力のある細胞を得ることができる。すなわち、両接合型を混合すれば、鞭毛先端の接触によっておこる細胞の凝集反応⁹⁾が見られる。この凝集した細胞群はやがて小さく崩壊し、時間のたつにつれて、2 個の細胞からなる接合対が多くできる。この接合対は各細胞の先端部で1本の原形質系の橋渡しによっ

* Contribution from the Biological Institute, Faculty of Science, Kobe University, Mikage, Kobe, Japan.

** The Biological Institute, Faculty of Science, Kobe University, Mikage, Kobe, Japan. (神戸大学理学部生物学教室)

* 無機塩培地: NH_4NO_3 , 250 mg.; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 175 mg.; K_2HPO_4 , 75 mg.; KH_2PO_4 , 175 mg.; $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 50 mg.; NaCl , 25 mg.; $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$, 10 mg.; Microelements (B, Mn, Co, Zn, Cu)²¹⁾; Dist. Water, 1 liter; Agar, 1.0%.

て連結されており、この形のままでおよそ6時間ほど泳ぎ、その後べん毛を失って両細胞は融合し、接合子となる⁴⁾。

両性の配偶子を試験管内に混合後、明暗いずれにおいても、およそ2時間半すれば、凝集した細胞群はすでに見られず、接合しうる細胞はすべて接合対を作ってしまうので¹⁰⁾、このときは一滴のヨウ素・ヨウ化カリウム液を落として細胞を固定し、顕微鏡下に接合対の頻度をかぞえれば、混合時における細胞の接合能力を推定することができる。本実験においては、接種、その他培養を暗所で短時間取り扱うばあいには、暗室内において、黄および緑のセロファンをまいた 10 W 螢光球からの弱光のかけで行なった。なお、両接合型を混合後、接合対数の測定までの2時間半は培養を完全暗黒内においた。

実験結果

1. 接合に対する光の促進効果

本実験中のすべての検査において、前記のようにして準備された藻は、照明されるまでもなく、すでに暗所においてある程度接合能力のあることを示した。しかし、Table. 1 に示されるように、光が与えられることにより、その接合能力はさらに高められることは明かである。また、ひとたび光をうけた細胞は暗所において混合されたばあいにも接合対を作りうる。しかし、光をうけた細胞を洗った場合には、その接合頻度は暗所において低く、再び照明をうけることにより高められる。このことは暗細胞が照明をうけることにより接合に有効な物質の生産が高められること、さらにそれが細胞外に排出されることを暗示するものである。

さて、暗細胞の接合能力は照明により高められるが、接合能力が最高に達するまでにどれほどの照明

時間が必要なのであろうか？ このことを知るために、各接合型の暗細胞を 2000 lux の螢光灯下に移し、時間を追って再び暗所に移して直ちに混合し、2時間半後にそのなかに作られている接合対の頻度をしらべた。Fig. 1 にその一例が示されているように、暗細胞は照明開始後わずか5分以内に、最高の接合能力を得ることができる。もっともこの場合、数回の実験において暗細胞の接合頻度は 2 ~ 50 % と、かなりのひらきを示したが、暗所での接合能力が低いときには照明された細胞の接合頻度もそれに応じて低くあらわれるのが通例であった。しかしいかなる場合にも、照明時間 30 分以内にはそのときの培養についての最高の接合能力を得るようになった。暗細胞の接合頻度についての各回の実験値に変動が見られる原因についてはまだよくわからないが

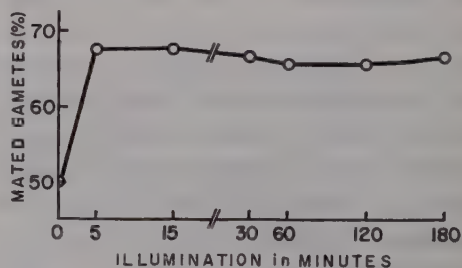


Fig. 1. Duration of illumination and increase in mating activity.

前培養のプレートにおける条件のわずかな差がこれに関係しているのかも知れない。この点はさらに検討を要すると思われる。なお、暗細胞における接合が、暗室内で短時間にうけた弱光の影響によるものでないことを確かめるために、完全暗黒下で実験をこころみしたが、その場合にもやはり接合が見られた。

Table 1. Effect of illumination on mating of *Chlamydomonas moewusii* var. *rotunda*.

Procedures	Mated gametes (%)
1. Mating of dark-cells in the dark.	2.4
2. Mating of cells illuminated for 1 hr.,	
(a) in the light.	40.0
(b) in the dark.	37.0
3. Illuminated cells washed and re-suspended,	
(a) in the light.	36.0
(b) in the dark.	3.5

また、細胞の暗期を 20 時間以上 48 時間まで長くしたばあい、この処理は細胞の運動能力を低下させ、したがって、暗所における接合をなくすことがわかり、またそのような細胞では照明を与えても細胞の運動が回復するまでかなりの時間を要し、同時に接合能力もきわめて低いという結果となった。

2. 暗細胞の接合におよぼす照明培養上清液の効果

前述の実験において、暗細胞が光をうけたときになんらかの接合有効物質が培地中に排出されると考えられた。そこで、実際にこの物質の存在を知る目的で、照明培養 1 時間の上清液をとり (3000 rpm, 10 min), これを暗細胞の培養に加えて、その接合におよぼす効果について調べることにした。なお、接合試験のために用いた細胞はすべて液体培地で 2 回洗った暗細胞である。Table 2 には明暗両培養について種々の組み合わせで調べられた接合能力、および照明培養上清液の活性度を調べた実験結果が総括

して示されている。このさい、配偶子形成のための培地として、M-N のほか、通例クラミドモナス用培地に用いられているクエン酸ソーダ (0.5 g/l), あるいは酢酸ソーダ (1 g/l) を加えたものについて行なった実験結果もあわせて示されている。この実験からわかるように、(+), (-) いずれかの接合型が照明されて、高い接合能力を得ておれば、反対の接合型をもつ暗細胞と混合したときに、暗所でさかんな接合がおこること、さらに、(+), (-) 両者とも光をうけることにより接合有効物質を出し、これが非特異的に暗細胞の接合をうながすらしいことがわかる。また、新鮮な上清液を室温で明暗いずれに放置したもので、1 時間後にはすでにその活性を失っている。一方、アンブルにつめて 100° 30 分の処理をおこなったものでは逆に接合を阻害するような結果になった。したがって、この有効物質はきわめて不安定なものようである。

Table 2. Effect of the supernatant of illuminated culture on dark mating in *Chlamydomonas moewusii* var. *rotunda*.

Combination in the mixture	Mated gametes (%)		
	Exp. 1	Exp. 2	Exp. 3
1. D(+) × D(-), 1 ml. each, plus 2 ml. medium*, a) placed in the light.	54.8	70.8	78.2
b) placed in the dark.	28.0	51.5	42.4
2. L(+) × L(-), 1 ml. each with medium* 2 ml.	59.8	—	—
3. L(+) × D(-), 1 ml. each with medium* 2 ml.	58.7	64.7	—
4. D(+) × L(-), 1 ml. each with medium* 2 ml.	58.9	62.8	—
5. D(+) × D(-), 1 ml. each, plus 2 ml. supern. L(+) supern. 2 ml.	54.3	73.3	—
6. D(+) × D(-), 1 ml. each with L(-) supern. 2 ml.	47.7	64.3	—
7. D(+) × D(-), 1 ml. each with mixed supern. L(+)/L(-), 2 ml.	51.3	73.0	74.0
8. D(+) × D(-), 1 ml. each with mixed supern. D(+)/D(-), 2 ml.	28.3	—	34.2
9. D(+) × D(-), 1 ml. each with mixed supern. L(+)/L(-), 2 ml. kept at room temp. for 1 hr. in the light.	—	—	36.8
10. D(+) × D(-), 1 ml. each with mixed supern. L(+)/L(-), 2 ml. kept at room temp. for 1 hr. in the dark.	—	—	35.0
11. D(+) × D(-), 1 ml. each with mixed supern. L(+)/L(-), 2 ml. treated by 100° for 30 min.	—	—	13.2

All the mixtures except 1-a) were placed in the dark until % mating was counted.

* medium: M-N with citrate in Exp. 1, with acetate in Exp. 2, and without any addition in Exp. 3. D: dark cells. L: illuminated cells. (+): (-): mating type. —: not examined.

Fischer^{11,12)}によると、大腸菌 K-12 株の接合は、その呼吸基質となるような物質、たとえば, glucose, aspartic acid, あるいは Krebs cycle 上の有機酸を与えることにより、頻度が高められる。いいかえれば、接合はエネルギー供給を必要とする現象である。そこでクラミドモナスについて Table 3 に示されるような物質を与え、暗所におけるその接合能力を調べてみた。培地 M-N に投与物質の最終濃度が 0.1% になるように、プレートに増殖したそれぞれの接合型の細胞を移し、暗所に 20 時間保ち、その後同一処理区の両接合型を暗所で混合した対象区としての培養は、M-N に懸濁したものを、20時間暗所に保ち、一部に暗所で混合し、他は 1 時間光を与えた後に暗所で混合したものである。この結果、これら投与物質のなかには多少とも暗所における細胞の接合能力を高めると思われるものがあったが、光の効果に代わりうるものは見出されなかった。

Table 3. Mating activity of dark cells obtained in organic compounds.

Compounds	Mated gametes (%)
M-N (illuminated)	84.0
M-N (dark)	28.3
M-N + Acetate (dark)	36.9
" + Citrate (")	22.1
" + Fumarate (")	41.5
" + Succinate (")	36.2
" + Malate (")	44.5
" + Pyruvate (")	13.2
" + Glycerin (")	39.0
" + Glycerophosphate (dark)	45.2
" + Glucose (")	43.8
" + Sucrose (")	24.3
" + Yeast extract (")	1.7

考察ならびに結論

Ch. moewusii var. *rotunda* では、その(一)接合型の配偶子から揮発性の走化性物質がすでに出され、(十)接合型の配偶子がこれにひきよせられることが筆者により報告されている^{9,10)}、今回の実験の結果、この藻の配偶子の上清液には、さらに別のはたらしをもつ接合有効物質があるように思われたが、こ

れは、前記の走化性物質と異なり、熱に不安定であり、その作用が(+)、(−)いずれの接合型からも検出できたという点において、両者は同一物ではないと思われる。また、その有効物質の作用様式をみれば、Moewus により報告された *Ch. eugametos* のそれ¹³⁾とは異なっている。Moewus の実験は疑問を残したままになっているが¹⁴⁾、近時 Förster & Wiese^{6,15)}も *Ch. eugametos* で "Gamon" を見出したように、クラミドモナスの生殖現象にはなんらかの作用物質が関係していることは否定できないであろう。

これまでに研究されてきたクラミドモナスのうちで、その大部分のもの^{1,5,16-20)}が有性生殖のために光を必要とするといわれるが、他方、*Ch. minutissima*²¹⁾、*Ch. eugametos* の雌株⁶⁾、および *Ch. reinhardi*³⁾は暗所でもある程度接合しうることが知られている。*Ch. moewusii* var. *rotunda* では、その生長過程のある段階において、あるいは栄養条件の変化によって、細胞がなんらかの特殊な生理的状态に到達したときには、暗所でも配偶子となりうる能力をそなえており、光がさらに接合能力を高めるようである。Lewin^{2,5)}によれば、*Ch. moewusii* の接合に対する光の作用波長は葉緑素の吸収スペクトルに平行している。同様のことは、Förster²²⁾による *Ch. eugametos* についての実験からもうかがえる。すなわち、接合能力の産生は光合成に関係した代謝過程に依存しているもののようである。

種々の炭水化物のなかには、*Ch. moewusii* var. *rotunda* の暗所接合をわずかながらも高めると思われるものもあった。おそらく、これらの物質は呼吸基質としてこの藻に利用されうるものであろう。呼吸の結果生じたエネルギーが接合のために利用されうことは当然考えられる。それならば一方、光を与えられることによりおこるであろう photo-phosphorylation²³⁾により作られるエネルギーで、照明細胞の接合能力がさらに高められることも想像できよう。

本研究にさいし、絶えずお励ましいたっている神戸大学広瀬弘幸教授ならびに京都大学今村駿一郎教授に深く感謝いたします。

文 献

- 1) Klebs, G., "Die Bedingungen der Fortpflanzung bei einigen Algen und Pilzen", G. Fischer, Jena (1896).
- 2) Lewin, R.A., In "Sex in Microorganisms" ed. by Wenrich, D.H., A. A. A. S. Symp. : 100 (1954).
- 3) Sager, R., and Granick, S., J. Gen. Physiol. **37** : 729 (1954).
- 4) Tsubo, Y., Bot. Mag. Tokyo **69** : 1 (1956).
- 5) Lewin, R. A., J. Gen. Microbiol. **15** : 170 (1956).
- 6) Förster, H., and Wiese, L., Z. Naturforschg. **9 b** ; 470 (1954).
- 7) —, and —, ibid. **9 b** : 548 (1954).
- 8) Tsubo, Y., J. Protozool. **8** : 114 (1961).
- 9) —, Bot. Mag. Tokyo **70** : 327 (1957).
- 10) —, ibid. **74** : 442 (1961).
- 11) Fischer, K. W., J. Gen. Microbiol. **16** : 120 (1957).
- 12) —, ibid. **16** : 136 (1957).
- 13) Moewus, F., J. wiss. Bot. **86** : 753 (1938).
- 14) Ryan, F. J., Science **112** : 470 (1955).
- 15) Förster, H., and Wiese, L., Z. Naturforschg. **11 b** : 315 (1956).
- 16) Bold, H., Bull. Torrey Bot. Club **76** : 101 (1949).
- 17) Hutner, S. H., and Provasoli, L., In "Biochemistry and Physiology of Protozoa" ed. by Lwoff, A., Academic Press, N.Y. **I** : 27, (1951).
- 18) Moewus, F., Arch. Protistenk. **80** : 469 (1933).
- 19) Smith, G. M., Science **108** : 880 (1948).
- 20) —, In "Plant Growth Substances" ed. by F. Skoog, Wisconsin Univ. Press, Madison : 315 (1951).
- 21) —, Amer. J. Bot. **33** : 625 (1946).
- 22) Förster, H., Z. Naturforschg. **12 b** : 765 (1957).
- 23) Arnon, D. I., Whatley, F. R., and Allen, M. B., J. Amer. Chem. Soc. **76** : 6324 (1954).

Summary

When light-grown plate culture of *Chlamydomonas moewusii* var. *rotunda* was flooded with liquid medium and kept in the dark, gamete-cells were obtained. Mating in such dark cells was poor; the activity, however, was increased by illuminating the cells. If the illuminated cells, of any mating type were mixed together in the dark with dark cells of the opposite mating type, the yield of mating pairs was as high as that obtained in a mixture of illuminated cells and the illuminated partners.

Furthermore, it was demonstrated that the supernatant of illuminated cultures enhanced non-specifically the mating activity of dark cells. The active principle differed from that for the chemotaxis reported (Tsubo, Y., 1957; 1961) in (1) that it was heatunstable, and (2) that it was detected in cultures of both mating types. Some substances on Krebs cycle seemed to increase the mating activity of dark cells, but illumination definitely gave a higher activity.

ヒガンバナ属の人工雑種の形態学的・細胞学的研究 I.

Lycoris sprengeri Comes. と *Lycoris straminea* Lindl. との雑種 F₁ について

竹 村 英 一*

Ei-ichi TAKEMURA*: Morphological and Cytological Studies on Artificial Hybrids in the Genus *Lycoris* I.

On the F₁ Hybrid between *L. sprengeri* Comes. and *L. straminea* Lindl.

1961 年 7 月 21 日受付

ヒガンバナ属の細胞学的研究は、個々の植物については、西山 (1928¹⁾), 竹中 (1930²⁾), 稲荷山 (1931³), '32⁴), '33, '37⁵), '39⁶), '51⁷), 佐藤 (1938⁸)), Bose (1957, '58) などによって詳細に行なわれているが、この属の人工雑種については、わずかに小山 (1954¹¹), '55¹²)), の報告があるにすぎない。ヒガンバナ属では、核学的な問題、開花期のずれ、結実期における栄養などのために、雑種を得ることがきわめて困難であり、たとえ結実しても不稔の種子が多いこと、また播種から開花までに長年月を要することなどが、研究をこれまでにはばんだ主な理由と思われる。しかし、人工雑種がまったく得られないわけではない。

著者は、1951 年以来的の研究によって、10 種の人工雑種を作り出し、近年ようやく二、三の植物が開花した。今回は、その一つである *L. sprengeri* Comes. と *L. straminea* Lindl. との間の人工雑種について記載する。

材料および方法

親植物として用いた *L. sprengeri* Comes. と *L. straminea* Lindl. とは、かつて、稲荷山教授が東京教育大学で研究に供したものをひきついたもので

* Botanical Institute, Faculty of Science, Tokyo University of Education, Otsuka, Tokyo, Japan. 東京教育大学理学部植物学教室

ある。

これらの植物は、ともに稔性が高く、開花期もほとんど同時期であるから、正逆交雑も容易である。交配は、午前中に行ない、普通の人工交配のほかに柱頭にしよ糖溶液 (0.2 M) を噴霧して受粉する方法も試みた。結実・採種は、一部は徳川・江本法¹⁰⁾により、室内で行なったが、大部分は圃場で行なった。体細胞分裂の場合の染色体の観察には根端細胞を用いた。8-Hydroxyquinoline (0.002 M) で4時間前処理したものを、Carnoy 液 (3:1) で15分間固定し、1 N 塩酸で5分間加水分解し、Feulgen 染色または acetic orcein 染色をしたのち、押しつぶし法によって観察した。還元分裂については、花粉母細胞を用い、Bonn 液で固定し、パラフィン法で切片 (厚さ 20~25 μ) をつくり、ハイデンハイン氏鉄明礬ヘマトキシリン法で染色して観察した。

観 察

1. 交 配

結果は第1表に示したとおりである。表中、得られた種子の型 a, b, c は、Fig. 1 の a, b, c にあたる。a は、直径 7 mm 以上で、黒い種皮をかぶっているもの、b は、種子の直径が 7 mm 以下のもの、および種子が未熟で種皮の白いもの、c は、胚はみとめられるが、その他の組織は未熟なもの、不稔のものなどを含めてある。得られた種子は、滅

Table 1. Results obtained by intra- and interspecific crosses in the genus *Lycoris*.

Combination (♀) (♂)	Number of pollinations	Type of seed			Hybrid F ₁ obtained
		a	b	c	
<i>L. sprengeri</i> × <i>L. sprengeri</i>	233	186	69	40	56
<i>L. straminea</i> × <i>L. straminea</i>	57	11	1	12	3
<i>L. squamigera</i> × <i>L. squamigera</i>	101	0	0	0	0
<i>L. sprengeri</i> × <i>L. straminea</i>	161	23	12	39	20
<i>L. straminea</i> × <i>L. sprengeri</i>	102	3	4	3	1

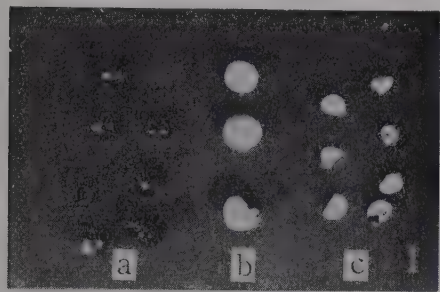


Fig. 1. Seeds of *Lycoris* (ca. × 1).
a : fertile and mature seed.
b : fertile and immature seed.
c : sterile and immature seed.

菌土を入れた植木鉢にまき、露天で植物を育てた。

2. 外部形態

交雑で得られた人工雑種は、花・葉・鱗茎とも、ほぼ両親の中間型を示すが、どちらかといえば *L. straminea* に近く、花は淡紅紫色である (Figs. 2, 3, 4, 10)。その他の形質については、第2表にまとめた。

3. 核学的観察

親植物として用いた *L. sprengeri* と *L. straminea* との核学的な所見は、稻荷山 (1937⁶) の報告とまったく同様であった。すなわち、*L. sprengeri* は $2n=22$ で、多少長さの異なる棒形染色体 11 対からなる同質 2 倍体であり (Fig. 6 および 6a), *L. straminea* は $2n=16$ で、長さのやや異なる棒形染色体 5 対 (すなわち、棒形染色体 10 個) のほか、長大で棒形染色体のほぼ 2 倍の長さを持ち、中央部近くに紡錘糸付着点をもつ V 形染色体 3 対 (すなわち、6 本の V 形染色体) からなる同質 2 倍体である (Fig. 8 および 8a)。

これら両者間の人工雑種は、根端細胞では $2n=$

19 で、V 形染色体 3 個と棒形染色体 16 個とからなっている。3 個の V 形染色体は、*L. straminea* における V 形染色体と同様に、長大では棒形染色体の 2 倍の長さを持ち、その中央部近くに紡錘糸付着点がある。16 個の棒形染色体は、*L. sprengeri*, *L. straminea* に見られるように、形は同じであるが、長さは少しずつ異なっている (Fig. 7 および 7a)。これらの染色体は、長さ・形がそれぞれ両親のものと似ており、その数は両親の半数染色体の和に等しい。

まれに、染色体構成が、棒形染色体 18 個と V 形染色体 2 個とからなる $2n=20$ の細胞をもつ個体も観察されたが、大部分の個体は $2n=19$ であった。

体細胞分裂は、一般に正常である。

減数分裂における染色体の行動を観察すると、花粉母細胞の第一分裂前期 (接合期) には、おのおのの V 形染色体の両腕に、2 個の棒形染色体が 1 個ずつゆるやかに対合した 3 個の異形の三価染色体と、棒形染色体がたがい 2 個ずつ対合し 5 個の二価染色体が見られる (Fig. 12 よび 12a)。対合の状態は、あるものでは強く、あるものでは緩い。ときには対合することなく、一価染色体のままで残るものもある。すなわち、大部分は $3\text{III}+5\text{II}$ の対合であるが、 $3\text{III}+4\text{II}+2\text{I}$, $3\text{III}+3\text{II}+4\text{I}$, $2\text{III}+5\text{II}+3\text{I}$ などの対合も観察され、かなり不規則である (Table 3)。

第一分裂の後期では、さきに稻荷山 (1932⁴) がシロバナヒガンバナ (*L. albiflora*) で観察したと同じように、棒形染色体どうしからなる二価染色体はふつうの分裂をするが、V 形染色体 1 個と棒形染色体 2 個が対合した異形三価染色体では、1 個の V 形染色体が一方の極へ、2 個の棒形染色体が他の極へ移動する場合と、2 個の棒形染色体がそれぞれ分

Table 2. Comparison of the morphological characteristics among *L. sprengeri*, *L. straminea*, their F₁ hybrid, and *L. squamigera*.

Organ	Species	<i>L. sprengeri</i>	<i>L. straminea</i>	F ₁ hybrid	<i>L. squamigera</i>
Flower:					
Color of perigone		mauve	straw-colored	rose-pink	pale rose
Length of tepalsegs		6 cm.	10 cm.	10 cm.	10 cm.
Shape of tepalsegs		oblanceolate	long-oblanceolate	long and narrow oblanceolate	wide-oblanceolate
Diameter		5 cm.	7 cm.	7 cm.	8 cm.
Color of anther in bud		yellow	yellow	yellow (reddish)	yellow (reddish)
Length of scape		30 cm.	70 cm.	65 cm.	80 cm.
Color of scape		purplish green	green	light purplish green	light purplish green
No. of flowers in a scape		5—7	6—8	5—7	5—7
Flowering time		late August	late August	late July— late August	early August
Pollen		fertile	fertile	sterile	sterile
Seed		fertile	fertile	sterile	sterile
Leaf:					
Length		30 cm.	33 cm.	35 cm.	40 cm.
Width		1 cm.	2 cm.	2 cm.	3 cm.
Color		green	light green	whitish green	whitish green
Quality		tough and thick	soft and thin	soft and thin	soft and thin
Whole shape		linear	long ligulate	long ligulate	long ligulate
Shape of apex		obtuse	orbicular	orbicular	orbicular
Leaf period		Feb.—June	Feb.—June	Feb.—June	Feb.—June
Bulb:					
Shape		ovate	globose	intermediate	globose
Diameter		3.5 cm.	4.5 cm	4.7 cm.	5 cm.
Color		dark brownish black	dark brown	dark brown	dark brown

Table 3. Frequency of chromosome pairing at 1st metaphase in F₁ hybrid obtained from *L. sprengeri* × *L. straminea*.

Chromosome pairing	3 III +5 II	3 III +4 II +2 I	2 III +6 II +1 I	3 III +3 II +4 I	2 III +5 II +3 I	2 III +4 II +5 I	Total
Number	29	18	2	6	9	7	71
Rate (%)	41.1	25.8	2.8	8.4	12.6	9.8	100.0



れて両極へ向かい、V形染色体がいずれか一方の極に移動する場合とが観察された。とくに後者では、中期に2個の棒形染色体がV形染色体の両端に結合したまま両極に引かれるために、V形染色体が引きのばされている像がしばしば観察された。

花粉粒の核分裂は、材料が少なく、十分な観察ができなかったが、上述のような不規則な分裂のためか、不稔の花粉が多く、acetocarmine 染色で観察したとき、細胞内容が充実して核がみとめられるものと、内容が空虚で小形のものとがあり、その割合は Table 4 のとおりであった (Fig. 13)。

Table 4. Rate of functional pollen grains produced in artificial hybrid.

Grain \ Type	Full	Empty	Total
Number of grains	428	298	726
Rate (%)	58.9	41.1	100

論 議

今回得られた人工雑種は $2n=19$ であり、V形染色体3個と棒形染色体16個からなるが、これは両親植物である *L. sprengeri* (棒形染色体22個)の半数と *L. straminea* (V形染色体6個と棒形染色体10個)の半数との和である。また、染色体数のうえからだけでなく、この人工雑種の染色体の形および大きさについても、両親のそれらとほとんど同じである (Table 5)。

このことからすると、雑種の染色体19個のうち棒形染色体11個は *L. sprengeri* から由来し、残りの8個 (棒形染色体5個 + V形染色体3個) は

L. straminea からきたものと考えられる (Fig. 14)。

花粉母細胞においては、減数分裂の第一分裂で、8個の対合染色体をつくり、そのうち3個は、異形三価染色体であり、5個は二価染色体である。そして、3個の異形三価染色体は、*L. straminea* のV形染色体の両腕に *L. sprengeri* の棒形染色体がそれぞれ1本ずつゆるやかに対合したものであり、5個の二価染色体では、いずれも *L. straminea* の棒形染色体と *L. sprengeri* の棒形染色体とが1本ずつ対合してできたものである。

このように、すべての染色体に接合が行なわれることからすれば、*L. sprengeri* と *L. straminea* とは、核型がかなり異なるにもかかわらず、染色体の相同性はきわめて高いものと考えられる。

L. straminea に由来するV形染色体の両腕に、*L. sprengeri* に由来する棒形染色体が1本ずつ対合して、異形三価染色体を形成する事実は、V形染色体1個は棒形染色体2個と等価なことを示している。

稲荷山 (1951⁷⁾) は、このような異形三価染色体が、*L. albiflora* (シロバナヒガンバナ)、*L. aurea* (ジョウキラン) において、また異形四価染色体が、*L. squamigera* (ナツズイセン) において形成されること、および *Lycoris* 属各種の核型の比較研究に基いて、*Lycoris* 属に見られるV形染色体は、棒形染色体が2個ずつ癒合して生じたものであろうと述べているが、この見解は、今回の人工雑種における異形三価染色体の観察によって、一層強く支持されるであろう。

また、稲荷山 (1939⁸) は、*L. squamigera* (ナツズイセン) が外部形態的には *L. sprengeri* と *L. straminea* との特徴を合わせ持ち、核学的にも

Figs. 2-5. Flowers (ca. $\times 2/5$).

2: *L. sprengeri*, 3: *L. sprengeri* \times *L. straminea*, 4: *L. straminea*, 5: *L. squamigera*.

Figs. 6-9, 6a-9a. Somatic chromosomes in root tip cell (ca. $\times 700$).

6 and 6a: *L. sprengeri* ($2n=22=22R$), 7 and 7a: *L. sprengeri* \times *L. straminea* ($2n=19=3V+16R$), 8 and 8a: *L. straminea* ($2n=16=6V+10R$), 9 and 9a: *L. squamigera* ($2n=27=6V+21R$). Arrow indicates V-shaped chromosome.

Figs. 10 and 11. Foliage and bulbs (Scale 30 cm).

10. Left, *L. sprengeri*; middle, *L. sprengeri* \times *L. straminea*; right, *L. straminea*.

11. Left, *L. sprengeri* \times *L. straminea*; right, *L. squamigera*.

Figs. 12 and 12a. Chromosome pairing in pollen mother cell of the artificial hybrid (ca. $\times 700$). Diakinesis. 3 III +5 II .

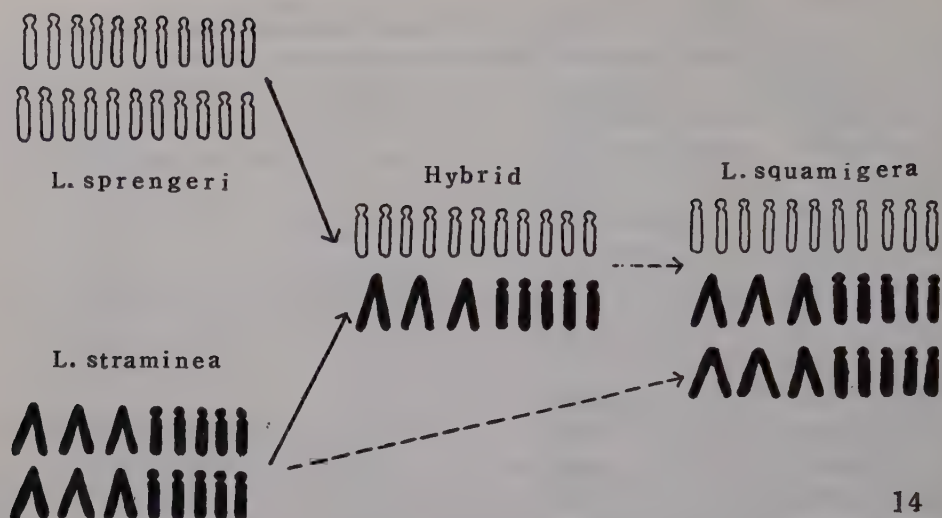
Fig. 13. Pollen grains of the artificial hybrid (ca. $\times 600$).

Table 5. Relative length of chromosomes (in μ). Numerical figures in a brace denote the arm-length of V-shaped chromosome. Each value is based on an average of 10 mitotic figures in each plants.

<i>L. sprengeri</i>		<i>L. straminea</i>		<i>F₁ hybrid</i>		<i>L. squamigera</i>	
I ₁	12.9	V ₁	24.2 {13.6 10.6	V ₁	26.8 {13.7 13.1	V ₁	30.9 {16.2 14.7
I ₂	12.5						
I ₃	12.4	V ₂	23.0 {12.0 11.0	V ₂	24.6 {12.5 12.1	V ₂	29.1 {14.6 14.5
I ₄	12.1						
I ₅	11.9	V ₃	22.9 {12.0 10.9	V ₃	23.7 {12.2 11.5	V ₃	25.0 {12.5 12.5
I ₆	11.6						
I ₇	11.2	V ₄	21.0 {11.3 9.7	I ₁	12.1	V ₄	24.9 {13.6 11.1
I ₈	10.8			I ₂	11.2		
I ₉	10.5	V ₅	20.3 {10.6 9.7	I ₃	10.7	V ₅	24.4 {13.5 10.9
I ₁₀	10.5			I ₄	10.7		
I ₁₁	10.5	V ₆	19.2 {10.1 9.1	I ₅	10.5	V ₆	20.4 {11.2 9.2
I ₁₂	10.3			I ₆	10.5		
I ₁₃	10.2	I ₁	12.3	I ₇	10.3	I ₁	11.7
I ₁₄	10.0	I ₂	12.1	I ₈	10.2	I ₂	11.4
I ₁₅	10.0	I ₃	11.9	I ₉	10.2	I ₃	10.6
I ₁₆	9.7	I ₄	11.8	I ₁₀	10.0	I ₄	10.0
I ₁₇	9.3	I ₅	11.6	I ₁₁	10.0	I ₅	9.9
I ₁₈	9.2	I ₆	10.0	I ₁₂	9.9	I ₆	9.6
I ₁₉	9.1	I ₇	9.9	I ₁₃	9.5	I ₇	9.5
I ₂₀	9.1	I ₈	9.8	I ₁₄	9.3	I ₈	9.3
I ₂₁	9.0	I ₉	9.0	I ₁₅	9.1	I ₉	9.3
I ₂₂	8.2	I ₁₀	8.5	I ₁₆	8.2	I ₁₀	9.0
						I ₁₁	9.0
						I ₁₂	8.8
						I ₁₃	8.8
						I ₁₄	8.8
						I ₁₅	8.6
						I ₁₆	8.6
						I ₁₇	8.3
						I ₁₈	8.2
						I ₁₉	7.9
						I ₂₀	7.9
						I ₂₁	7.7
Total		231.0		237.5		347.4	

V形染色体 6 個と棒形染色体 21 個とを持つことから、*L. sprengeri* の正常な半数配偶子（棒形染色体 11 個）と *L. straminea* の倍数配偶子（V形染色体 6 個と棒形染色体 10 個）との接合による異質三倍体で、外部形態が *L. straminea* の方により多く似ているのは、*L. straminea* のゲノムを 1 個余

分にもつためであると考えた (Figs. 5, 9, 9a, 11).
今回作り出された人工雑種は、*L. sprengeri* の半数配偶子（棒形染色体 11 個）と、*L. straminea* の半数配偶子（V形染色体 3 個＋棒形染色体 5 個）とが接合してできた異質二倍体であり、*L. squa-*



14

Fig. 14. Diagrammatic representation showing karyotypical relationships in the artificial hybrid (*L. sprengeri* \times *L. straminea*) and *L. squamigera*.

migera (ナツズイセン) にくらべて *L. straminea* のゲノムが1個だけ少ないものである。また、外部形態的にも *L. sprengeri* と *L. straminea* の中間型を示し、しかもナツズイセンにも似ている。つまり、二倍体のナツズイセンとみなされる (Fig. 14)。

Bailey (1916, '35⁹), Traub and Moldenke (1949) らは, *L. squamigera* Maxim. (ナツズイセン) の一変種 *var. purpurea* Hort. という植物を記載し, このものはナツズイセンの二倍体植物であり, 稔性があると述べている。著者の得た人工雑種は不稔であるから, Bailey らの記載した植物とは異なるものである。Bailey らのいうナツズイセンの二倍体植物は, おそらく, *L. sprengeri* の一品種であろうと思う。すなわち, 著者が栽培した *L. sprengeri* のなかには, 花被の先端が丸味をおび, しかもこの部位の色の青味が少ない。外部形態的には, 著者の作った人工雑種やナツズイセンによく似たものがあり, これには稔性がある ($2n=22=22R$)。そしてこれらの特徴は Bailey らの記載に適合しており, この植物が *L. squamigera* Maxim. *var. purpurea* Hort. であろうと思われる。

摘 要

1. *L. sprengeri* と *L. straminea* との人工雑種を作り出して, その形態学および細胞学的特徴を調べた。

2. 人工雑種は, 外部形態的には両親植物の中間型を示すが, どちらかといえば *L. straminea* に近似である。

3. 核学的には根端細胞において, $2n=19=3V+16R$ を示し, 異質二倍体で, 染色体数は両親植物の半数染色体数の和である。

4. 花粉母細胞の第一分裂前期において, V形染色体の両腕に棒形染色体がそれぞれ1個ずつ対合した異形三価染色体を形成する。このことは, ヒガンバナ属に見られるV形染色体は棒形染色体2個に相当し, 棒形染色体の癒合によって生じたという稲荷山の見解を裏付けるものである。

5. この人工雑種は, 外部形態および染色体構成の上から, 異質三倍体である *L. squamigera* (ナツズイセン) の二倍体にあたるものと見なされる。

この研究は, 奈良学芸大学長, 稲荷山資生博士の懇篤なご指導と有益な助言のもとに行なわれたものであり, 先生に深く感謝の意を表します。

文 献

- 1) Nishiyama, I., Bot. Mag. Tokyo 42 : 509 (1928). 2) Takenaka, Y., Jour. Chosen Nat. Hist. Soc. No. 10:54 (1930). 3) Inariyama, S., Bot. Mag. Tokyo 45 : 11 (1931). 4) —, ibid. 46 : 426 (1932). 5) —, Sci. Rep. Tokyo Bunrika Daigaku, Sec. B, 3 : 95 (1937). 6) —, Rep. Jap. Sci. Cong. 14 : 636 (1939). 7) —, Sci. Rep. Tokyo Bunrika Daigaku, Sec. B, 7 : 75 (1951). 8) Satô, D., Cytologia 9 : 203 (1939). 9) Bailey, L. H., Stand. Cycl. Hort. II : 1933 (1935). 10) Tokugawa, Y., and Emoto, Y., Bot. Mag. Tokyo 44 : 236 (1930). 11) Koyama, M., Ann. Rep. of Doshisha Women's Coll. No. 4:128 (1954). 12) —, ibid. No. 6:285 (1955).

Summary

1. Morphological and cytological studies were carried out on the F_1 plants raised from the cross, *Lycoris sprengeri* Comes. ($2n=22=22R$) \times *L. straminea* Lindl. ($2n=16=6V+10R$).
2. The outer appearance of the F_1 hybrid was found to be intermediate between both parent plants, while it resembled more or less closely the pollen parent, *L. straminea* (Figs. 2-4, 10).
3. The chromosome number in somatic cells of the F_1 plant was $19=3V+16R$, i. e., the sum of the numbers in gametic cells of both parents (Figs. 6-9, 6 a-9 a).
4. At the 1st metaphase in microsporogenesis of the F_1 plants, 3 heteromorphic triplets and 5 bivalents have been commonly observed (Figs. 12, 12 a).
5. The F_1 plants were shown to be completely sterile in all selfing experiments.
6. From the number, shape and behavior of the chromosomes in meiotic division, it is suggested that this artificial hybrid seems to be adiploid form of *L. squamigera* Maxim. (Figs. 5, 9, 9 a, 11).
7. The synaptic behavior between V-shaped and rod-shaped chromosomes in this hybrid seems to support the Inariyama's view that in the genus *Lycoris* one V-shaped chromosome is equivalent to two rod-shaped ones, and, therefore, the former might have been derived by the fusion of the latter two.

Short Communication

Teruko NAKAMURA*, Hiroyoshi ISHII* and Toshio YAMAKI* : The Intracellular Localization of Native Auxin in *Avena* Coleoptile

中村輝子*・石井愼義*・八巻敏雄*： マカラスムギ幼葉鞘の細胞内における
オーキシンの分布について

Received November 15, 1961

As the first step of our course of study to find the master reaction of auxin, we have made researches about the intracellular localization of native auxin in *Avena sativa* (Victory No. 1) coleoptile.

About 10 mm. defoliated tips of *Avena* coleoptiles, which had been grown 20-30 mm. in darkness, were homogenized with cold sucrose-phosphate buffer of pH 5.5, and fractionated by differential centrifugation. Each fraction was acidified and extracted with ether and contaminations in this extract were removed by paper chromatography. The auxin activity in this extract was tested by *Avena* curvature test¹⁾. After the ether extraction, the residue of each fraction was adjusted to pH 9.5 and hydrolyzed 5 minutes at 120° and the hydrolyzates were acidified and extracted with ether. Each ether extract was purified by paper chromatography in these cases, too. One example of the results of our experiments is shown in Table 1.

Table 1.

Fractions of differential centri- fugation	<i>Avena</i> curvature in degree		
	Free auxin**	Bound auxin***	Control
1,000×g×10'	0.0°	0.0°	—
4,500×g×10'	0.0	?****	—
14,000×g×30'	0.0	0.0	—
100,000×g×30'	0.0	0.0	—
Supernatant	14.1	16.7	—
0.05 ppm IAA	—	—	18.6
Water	—	—	0.0

Fresh weight of the used coleoptile tips: 81 g. ** Ether extractable auxin.

*** Auxin which was extracted with ether after alkaline hydrolysis. ****

Low auxin activities were observed in 2 out of 8 experiments.

The localization of the native free auxin seems to be only in the supernatant, non-particulate cytoplasm and the cell sap, and the activity of native bound auxin is also found in the supernatant. Identification of the native auxin in this extract was carried out by paper chromatography. The Rf values of native free as well as native bound auxin were the same as that of IAA. Detailed report will be presented soon.

Reference

- 1) Shibaoka, H., and Yamaki, T., Bot. Mag. Tokyo **72**: 152 (1959).

* Biological Institute, College of General Education, University of Tokyo, Meguro, Tokyo, Japan. 東京大学教養学部生物学教室

本 会 記 事

第 26 回 (東京) 大会

日本植物学会第 26 回大会は、東京大学理学部、医学部および薬学部において、去る 10 月 13 日から 16 日まで、4 日にわたって開催された。当初参加申込みが 360 余名で、やや少なかったが、当日は 480 名の参加者を得て、予期以上の盛会となった。曇り勝ちの天気であったが雨とはならず、滞りなく終了、見学旅行当日は好天にさえ恵まれたのは、幸運であった。第三日目の昼食を懇親会としたのは新しい試みであったが、比較的若い人たちの参加が多かったのは成功であった。当日の参加申込みが多かったこと、見学取消しが予想外に多かったことなどで、事務的にやや混乱して、参加者に迷惑をおよぼしたことはまことに残念であった。

評議員会 (10 月 12 日午後 5 時—午後 9 時、学士会館本郷分館)

出席者：評議員 26 名 (欠席 4 名)、会長、幹事長より幹事 2 名、計 30 名。

報告：1. 役員移動。2. 会員状況。3. 図書の貸出状態および交換寄贈の状況。4. 昭和 36 年度会計中間報告。5. 昭和 37 年度予算。6. 植物学雑誌論文投稿および掲載状況。

以上の事項について会長および幹事長から報告があり、評議員全員が承認した。

議題：1. 昭和 37 年より植物学雑誌を年 12 回発行する件。

会長および幹事長より植物学雑誌は毎月発行を建前としているが、実際には 2 回合併号を出して年 10 回を発行している。しかし第 3 種郵便物の規定では、定期的休刊、合併号は認めないから、来年度よりできるだけ年 12 回発行したいむねの説明があり、これにともなう予算の変動などについての討論のち可決。

2. 図書処分件

会長および幹事長より、学会が寄贈をうけたり、かつて交換した図書のなかには全く会員に利用されず保管する価値のうすいものがあり、一方学会の書庫 (東京大学理学部付属植物園の書庫の一部を借用) は狭いのでこれらの図書を処分したいむねの説明が

あった。これに対し賛成意見が多く、14 日の総会に議題としてかけることになった。

3. 次期および次次期大会開催地の件

次期大会は名古屋大学において、次次期大会は岡山大学において開催することを内定した。

4. 総会における議長の件

従来総会には議長をおこななかったが、今回より議長をおきたいむね会長より提案があり、下斗米直昌氏を 14 日の総会の議長候補にきめた。

なお来年は創立 80 周年を迎えるので、この機会に名誉会員、特別会員、外国通信会員を推薦することが話題になり、来年 6 月までに各支部が資料を集めて適当と思われる人を本部に通知することを決定した。

総 会 (10 月 14 日午後 1 時—午後 2 時、東京大学薬学部記念講堂)

出席者 88 名。会長の挨拶ののち議長に下斗米直昌氏を選出し、ついで会長および幹事長より次の諸事項の報告があった。

1. 役員移動 (植物学雑誌昭和 36 年 4 月号掲載)

2. 会員状況 (昭和 36 年 9 月 10 日現在)

現在会員数：名誉会員 18 名、特別会員 23 名、外国通信会員 7 名、終身会員 55 名、通常会員 1290 名、計 1393 名。

会員移動：新入会 94 名 (昭和 35 年 10 月 11 日以降)、死亡 5 名、退会 11 名、除名 30 名、差引増加 48 名。

3. 図書の交換寄贈の状況

交換：国外受理 78、国外発送 72、国内受理 38、国内発送 36。寄贈：国外受理 11、国外発送 25、国内受理 48、国内発送 5。

4. 図書の貸出状況

年度	延貸出冊数	貸出をした人数
1956	90	31
1957	118	33
1958	114	44
1959	92	31
1960	106	29
1961 (9 月まで)	98	33

5. 昭和 35 年度会計決算 (植物学雑誌昭和 36 年

3月号に掲載)

6. 昭和 36 年度会計中間報告
7. 昭和 37 年度会計予算
8. 植物学雑誌刊行経過および予定

年度	収 載 論文数	総ペー ジ 数	
1954	45	306	(隔月刊)
1955	64	372	(年10回刊)
1956	97	602	(小倉記念号発行、会 費 900円に値上げ)
1957	77	438	(75周年記念号発行)
1958	62	444	
1959	65	488	
1960	84	508	
1961 1月号	9	50	(会費1200円に値上げ)
2月号	5	40	
3月号	10	70	
4月号	8	38	
5月号	12	74	
6月号	7	44	
7-8月号	9	52	
9月号	7	47	
10月号	6	40	
11-12月号	10	70	(予定)
1961年度計	83	525	(予定)

これらの報告事項は異議なく承認された。

ついで議事にはいり会長から次の議題が提出された。

1. 不利用図書処分件

10月12日に開かれた評議員会での説明および討議の様子が会長から報告され討論ののち、「処分図書のリストをつくり、この中で支部移管の希望があるものがあるかどうかについて支部に回答を求める。期限内に回答のないものの処分は本部に任せる」との案が会長より提出され賛成多数で議決した。

2. 次期大会開催地の件

次期大会は昭和37年10月中旬に名古屋大学において開くことを決定。なお次次期大会は岡山大学に内定した。

最後に会長と大会会長小倉謙氏とから挨拶があって総会を終わった。

通 常 講 演

分類・地理・形態

石川元助: トリカブト属植物とその毒矢文化圏

増田染一郎: 湿室培養によって分離した *Myxobacteria* の新種について

椿 啓介: 石狩川河水の汚染糸状菌について

信夫隆治・島田善夫・川戸峯子: Whirl を形成する放線菌の一新種 *Streptomyces griseovorticellatus* について

川戸峯子・信夫隆治: 放線菌の窒素源利用について
II. 炭素源に glucose を用いた場合の NO_2^- と NO_3^- について

米山 穂: A new heterothallic yeast, *Endomycopsis scolyti*

曾根田正巳: 海産魚類腸管中の酵母について

小林艶子: 羽状ケイ藻 *Ceratoneis arcus* Kütz の変異

熊野 茂・瀬戸良三・広瀬弘幸: カワモズク属の一新種 *Batrachospermum ectocarpum* Sirod. の種内の変異および他種との関係について

瀬戸良三・熊野 茂・広瀬弘幸: カワモズク科数種の *Chantransia* stage の比較

笠原和男: コンプ科の粘液腔道について

猪野俊平・西林長朗: ツルアラメの遊走子囊発生と遊走子形成

広瀬弘幸: 淡水産シオグサ科 *Basycladia* 属の一新種について

千原光雄: 緑藻ブラシノクラズス・アスクスの生活史とその類縁についての一考察

加崎英男: 日本新産属 *Lamprothamnium* (Characeae) について

岩崎尚彦: ジャジクモ科植物の生長点の分化と器官形成 VI. *Tolypella gracilis*

堀川芳雄・関 太郎: *Brotherella recurvans* (Mich.) Fleisch, ミヤマカガミゴケ (セン類) について

百瀬静男: シダにおける無配生殖と種の分化

増淵法之: コムギ分枝穂の形態学的研究

高木虎雄: ササ属の花の分類学的知見

鈴木貞雄: 関東・東北地方産ササ属, チシマザサ節の分類

小宮定志: 南アフリカ産 *Roridula* の解剖学的知見

桃谷好美: たんぱく質から見たカエデ属の類縁 (第2報)

豊国秀夫: リンドウ属の分割について

福島 博・入山陸子: 南極大陸問題岩露岩帯のケイ藻

福島 博・星野郁子: 南極大陸新南露岩帯のケイ藻

大野正夫: 北海道知床半島ポロモイ台地のケイ藻類

奥野春雄: 北海道における海成ケイ藻土

野田光蔵: 日本海佐渡島に発生する緑藻ヒトエグサ (*Monostroma*) について

越智春美: 日本およびその近接地域産タマゴケ科セン類の分布について

三木 茂: 遺体フロラからみた邦産水生植物

粉川昭平: ミツガシワの過去と現在の分布

里見信生: 塩素酸カリの抗毒性による本州内外帯植物の判別

細胞・遺伝

木村劫二・角谷啓作: ふたたびウイングソヒトヨの三核性子実体について

竹村英一: ヒガンバナ属の人工雑種 (第5報)

向川信一: トウモロコシの混合受粉

武丸恒雄: 帽菌類におけるヘテロカリオン形成

坪 由宏: ストレプトマイシンにより誘発されるクラミドモナスの葉緑体変異について

奥田正男・柳島恒彦・高田英夫: 合成界面活性剤による酵母の呼吸欠損変異の誘起

竹中 要: 染井吉野の起源の決定と原産地の推定

左貝アイ子: 植物細胞のオスミウム固定についての電子顕微鏡の研究

上野実朗・北口貞夫: 電子顕微鏡によるヒツジグサ科花粉膜の微細構造

村上 悟: オオムギの根の色素体の prolamellar body (ラメラ形成体)

村上 悟: 葉緑体の grana lamellae の微細構造

遠山 益・植田利喜造: キャベツ葉の色素体の発達と構造に関する電子顕微鏡の研究

大隅正子・湯浅 明: 電子顕微鏡による有色体の研究

川松重信: アカウキクサの根のプラスチッドについて

湯浅 明: シダ植物の細胞学的研究 XXXV. 色素体の自律性

辰野誠次: *Selaginella* 属の細胞学的研究 II

藤原悠紀雄: *Aster* 属の核型分析 (第7報)

竹本貞一郎: ニガナ群の細胞学的研究 (続報)

神野太郎: *Polygonatum* (ナルコユリ属) 数種の染色体

下斗米直昌: 染色体数の異なる種間の自然雑種の細胞学的研究

茅野 博: ホウチャクソウにおける染色体不対合と非減数花粉粒の形成

松浦 一・岩淵雅樹: NaCl, KCl, CaCl₂ 処理によって誘発された減数分裂の異常

松浦 一・谷藤茂行・岩淵雅樹・金沢 甫: 還元分裂における半染色分体組換え像の出現について

大野林二郎: センブリ (*Swertia japonica*) 抽出液による花粉母細胞分裂の異常

劉 逸民: オオバナノエンレイソウの花粉母細胞分裂における高温処理の影響

加藤一男: 中間期における染色体の行動

植田勝巳: 8 ミリ映画によるムラサキツユクサの細胞の有糸分裂の観察

太田次郎・岡田順子: 変形体の振とう培養について
黒田清子・神谷宣郎: 細胞外に遊離した原形質滴の行動

加藤幸雄: シダの初期前葉体における細胞の単離

藪野恭三・清水 晃: 遠心処理したヒメフラスモ節間細胞について

中沢信午: 有極性原形質分離

八戸正夫: ユキノシタの葉の表皮細胞の原形質分離時の季節的变化と日変化について

高田英夫・山本 武: 高張ストロンチウムの溶液中の酵母原形質片の形成とその再生

山本 武・高田英夫: ストロンチウム高張環境における酵母原形質の顆粒化とその可逆性

米田芳秋: 酵母核の構造について

中村 威: 紡錘体および隔膜形成体の構造について

松浦 一: 自然における染色体切断の一例

松浦 一・武久 慎: *Trillium* の meiotic meta-phase I の染色体構造におよぼす EDTA の効果

馬場三吾: カルス形成過程における mitotic index の変化と二, 三の酵素活性の変化

松浦 一・佐保 貴・谷藤茂行・岩淵雅樹: X線誘起染色体異常におよぼす ATP と DNA の効果

松浦 一・佐保 貴・谷藤茂行・岩淵雅樹: X線誘起染色体異常におよぼす Mitomycin C の効果

山崎典子・水野忠款：コアツモリ染色体における加水分解の時間とフォイルゲン反応との関係について

山岸英夫：*Hydrodictyon reticulatum* の生長にもなう螢光染色性の変化

新家浪雄： ^3H -thymidine の細胞核への取り入れ

吉田吉男：葉緑体の働きにおよぼす細胞核の相関性

生理・生化

藤田善彦・服部明彦：フィコビルン色素の暗生成過程における光化学反応活性の変化について

加藤 栄・高宮 篤：葉緑体銅たんぱく Plastocyanin に関する諸光化学的反応について

千葉保胤・岡山繁樹：葉緑体の酵素処理と、その光化学活性

渡会彰彦：葉緑体の褪色と螢光

千葉保胤：葉緑体中の螢光物質について

菅原 淳・千葉保胤：葉緑体への P^{32} のとりこみ

IV. 光照射によるリビド分画へのとりこみの増加

宮地重遠：ホウレンソウ緑葉中におけるポリリン酸

西田晃二郎：光照射下に ^{14}C -glucose, 1,5- ^{14}C -citrate から放出される $^{14}\text{CO}_2$ について

野口市夫：葉緑体中に含まれるクェルセチン分解酵素について

藤茂 宏・和田善徳：高等植物の光合成能の消長に関する研究（第二報）

須藤俊造：アサカサノリ類の生長、成熟などに対する日長効果

杉野 守：アルビノコムギの日長反応

江刺洋司：シュウカイドウの短日反応に見られる $\text{FR} \Rightarrow \text{Blue, Green}$ および Red の可逆性について

滝本 敦・内藤佳之：種々の波長光下で育成したアサガオの日長感受性

木村和義・滝本 敦：低温によるアサガオの花芽形成

丸重啓二・丸重靖子：アサガオにおける花芽分化とたんぱく質分化

寺岡 宏：春化处理コムギ胚における窒素代謝

上坪英治：周回型原形質流動の向きについて

阿部重美：原形質運動と -SH

高沖 武：根の吸水機構に関する研究（I）

神谷宣郎・田沢 仁・足立堯子：フラスモ節間細胞の透過性

田沢 仁・足立堯子：細胞横断滲透法によるフラス

モ細胞のアルコールにたいする透過性の決定

永井玲子・田沢 仁：ヒメフラスモに見られる光電反応とイオン吸収

玉井直人・西田晃二郎：光合成産物の根への転流におよぼす光の影響

賀来章輔：植物組織の凍結曲線の分析（4）

衣川堅二郎：キノガサタケの生長について II. 特に pH との関係

今井百里江子：絶対好稠性を示す一新糸状菌について（第2報）

松下亀久：TMV の増殖に関する研究 そのIII

藤野正義：気孔の開閉と ATP, ATP-ase

巖佐耕三：セン類 (*Funaria hygrometrica*) の原糸体培養（予報）

内田 昭・芦田譲治：酵母の突然変異に対する銅の効果

荒勝 豊：含銅培地上での酵母の変異誘起における初期培養条件の意義

瀬野悍二・芦田譲治：酵母の銅耐性と細胞の銅の取込み

庄司善哉・倉石 衍：アデニン要求性酵母の増殖異常

三戸信人・柳田友道：酵母細胞の密集状態における生理的变化

鳥山英雄：オジギソウの細胞の生理学的研究（第15報）—葉柄の節部の基本構造について—

須田省三：オジギソウの刺激物質

柴岡孝雄：オジギソウの興奮性細胞における伝導

照本 勲：マリモの凍害と多価アルコール

渡辺 篤・清原千里：地衣、苔およびソテツと共生するラン藻について

林 克巳：トウキビとコムギの芽ばえの生長におよぼすビタミン類の影響

堀 武義：マツバボタンの花の開閉におよぼす光、熱の効果

武田幸作・林 孝三：パンジーの紫色色素について

柴田万年：チューリップの一品種 Charles Needham の花のアントシアン

石倉茂行・林 孝三：ダイコンの赤色根皮の anthocyanin について

菊地正彦・中原正城：*Penicillium islandicum* Sopp. の紫外線照射株における色素産生の消長

三井清司：オオボウシバナのフラボノイド物質につ

いて

村上 進: リボングラスのポリフルクトサンについて

西沢一俊・尾崎八郎: 紅藻でんぶんの生化学的研究
入来義彦・三輪知雄: 緑藻細胞膜間粘質物の生化学的研究 (II). 緑藻 *Collinsiella* の細胞膜間粘質物について

武田 宏・三輪知雄: カワノリの細胞膜間質

森 祐二: 細菌細胞壁溶解酵素と protoplast

鈴木 昇・奥田 聰・鈴木 旺: *Azotobacter* のピ
リジンヌクレオチド

浅田弘治: 食塩高張培地における *Bacillus subtilis*
の RNA

中村 運: 酵母の重金属 Cross Resistance におけ
るリボ核酸代謝の意義

木村孝一・新田 毅: 高等植物葉緑体の RNA 抽出
法に関する考察

沢井輝男: *Candida amylase* のでんぶん質よりの
glucose 転移について

菊池忠寿・芦田譲治: 酵母銅耐性株のいおう代謝

内貴信夫: 酵母のメチオニン要求株を用いたいおう
代謝の研究

服部明彦・渡辺 篤: ラン藻 *Anabaena cylindrica*
の硝酸還元系について

黒田清子: 正常および腫瘍組織による組織発生の誘
導現象について

三木寿子: *Primula obconica* における花粉管の負
の屈性

田中 清: アカマツ花粉に含まれる酸性生長抑制物
質の発芽に伴なう量的変化

柴岡弘郎: カフェー酸の生長阻害作用

小林万寿男: 茎における不定根発根の抑制

末本雛子: 一粒小麦の左右性の方向決定に対するイ
ンドール酢酸の効果

柳島直彦: 呼吸欠損酵母にみられるオーキシンの細
胞伸長促進作用

和田俊司・長尾昌之: イネの子葉鞘中のインドール
酢酸酸化酵素阻害物質 (続報)

中村輝子・石井愷義・八巻敏雄: アベナ子葉鞘の細
胞内のオーキシン分布

岡上伸雄・江刺洋司・長尾昌之: ジベレリンによる
シュウカイドウの無性芽形成の抑制と促進作用に
ついて

加藤次郎: タケノコのジベレリン様物質について

村上 浩: ジベレリンの植物体内における変化

高橋憲子・師尾武子・八巻敏雄: タバコ種子の暗発
芽に対する数種の有機酸の作用と pH との関係

山本昌木: *Phytophthora infestans* (Mont.) De
Bary 菌胞子発芽の二型について

今堀宏三・巖佐耕三: ジャシクモの発芽と生長の環
境コントロール

河原 晨: ヒシモドキの発芽について

山田晃弘: トウゴマ発芽時にみられる CoA 量と
ATP 量

宮本義男: 微生物によるパラフィンおよびろうの分
解 (続報)

小野田哲夫・宇佐美正一郎: *Staphylococcus au-*
reus 青酸耐性菌の呼吸について

村山徹郎・芦田譲治: 銅耐性酵母の TCA サイク
ルについて

田中滋郎・宇佐美正一郎: サトウダイコンの根の呼
吸系について

熊谷孝美・宇佐美正一郎: トマトとイヌホオズキの
つぎ木による周縁キメラ雑種の呼吸について

佐々木喜美子: ベゴニアの葉の呼吸酵素について

辻 英夫・浜田秀男: イネ芽ばえのエネルギー代謝
形式変動の乾燥重量への反映

駒嶺 穆・服部静夫: ハッショウマメにおけるチロ
シンの代謝

生 態

中西 哲: 着生群落ナガスジイトゴケ群集について
堀川芳雄・岡本 香: 広島県の地質とスゲ属植物に
ついて

北川昌典: 鈴鹿山系におけるマツ型森林の発達 (第
一報)

南川 幸: 鈴鹿山脈ツブラジイ林の天然更新に関す
る考察

菅沼孝之: 福島県檜枝岐村の花沼湿原の植物群落
(予報)

沼田 真・小村登志子・大木 薫・林 一方: 遷移
からみた埋土種子集団の解析

高橋基生: 植生の特異分布ならびに生育に対する植
物生態学的研究 I. 温度変異に基づく植生異常
(第2報). 神津島における環境要因ならびに植生
の特異性とその御蔵島との比較

高橋基生: 同上 II. 偶然要因に基づく植生異常

(第1報). 伊豆諸島における植生分布を支配する環境要因と偶然性について

高橋基生: 同上 III. 水分経済または根系呼吸の破綻, あるいは養料失調に基づく植生異常(第1報).

奥羽地方日本海岸寄り諸高山における亜高山帯針葉樹欠除に対する生態学的見解

矢野悟道: 草原における植物地下器官の生態学的考察, 特に放牧地について

宮脇 昭・大場達之: 奄美群島の海浜植生

倉内一二: 伊勢湾台風の害と回復状況—塩風害と海岸林 III

延原 肇・小滝一夫: 塩沼群落の問題点

柳沢新一: 発育の法則について, 付・生態学的限界に対する考察

岩城英夫・翠川文次郎: 霧ヶ峰草原群落の現存量と生産構造に対する採草の影響

大島康行: 数種のササの耐陰性

樫村利道: プナ林植物の葉の日補償点

野本宜夫: プナ林の生産構造について

小林 弘・市村俊英: 河川底生藻類の光合成について

シンポジウム

話題 1 変異性の問題

10月14日 14.30~17.00

(1) 永井 進(奈良女子大・理・生): 酵母菌類の形質的安定性と不安定性

(2) 西岡泰三(都立大・理・生): ニガナの種内変異の細胞遺伝学的研究

(3) 山崎 敬(東大・理・植): ササ類の分化と環境

座長 原田市太郎(名大・理・生)

永井氏は, コウボ細胞が, いろいろの既知の要因または原因不明の要因によって, 形態的ならびに生理的に変異することを, 実例をもって説明し, とくに呼吸能力を失った変異菌について, その複雑な遺伝的行動を説明した。

西岡氏は, ニガナの特殊環境に生育するいろいろの亜種タカネニガナ, クモモニガナ, イソニガナ, ドロニガナ, タニガワニガナなどは, すべて二倍体

であり, これらが独立に倍数化して, 現在見られる変異を生じたものであることを, 核型の観察から考察した。

山崎氏は, ササ類を例として話題を提供した。はじめに *Veronica* 属における染色体変異と環境との関係について説明し, ついでスズタケ, ミヤコザサ, チマキザサ, チシマザサなどの異なる生活型をもつ種が, 日光の中宮祠, 戦場原, 湯本の3か所の環境条件の異なる場所に住みわけており, それぞれの渗透価, 含水率, 溶質量がその生育する環境に適合していることを指摘した。

ついで座長よりつぎのような所見がのべられた。以上3つの話題は微生物から高等植物におよび, またその変異も生理的変異から核型の変異, さらに生活型の変異にまでおよぶきわめて分散したものであるが, その底に流れている問題は, 環境によってどのような生理的ならびに生態的変異が生ずるかという点にあると思われる。またこれらの変異が遺伝的のものであるか, 永続変異といわれるものであるか, あるいは一時的のものであるかという点も問題になるが, いま述べられた例はいずれも遺伝的変異であると思う。これらを分類学の方ではどうとりあつかうか, これらの変異がどのようにして生じたものであるかの問題やその他のいろいろの問題について活発な論議を希望する。

終わって討論にうつり, つぎのような意見がのべられた。コウボに見られるような生理的変異を分類の単位としてみとめるべきかどうかについて疑問が出された。たとえばトリカブトにビールスがついたようなばあい, 別の種とするのはどうか。これに対しては, 系統を主として考える分類の立場としてはこのような一般的な変異は分類の単位とは考えないが, 古いものではかならずしもそうっていないものがあるという意見がのべられた。この問題についてはさらに分類と命名とは区別すべきものであることが強調された。すなわち分類という立場からは, これらのあらゆる点を考慮すべきであるが, 命名という立場では, 便宜的な面が強くなるので, この考慮と命名とは別問題である。たとえば分類学者は園芸の品種には命名しないが, 園芸家はそれが必要であるので, 別の命名規約によって命名している。この見解は上にあげたいろいろの変異に対する分類学からの観念を明確に表現したものといえよう。後半

においては主としてササについて論議された。北海道ではササの生活型と雪との関係は話題提供者の様子とは異なるという意見があり、諸データの測定に用いた葉は、いずれも冬、雪の中から採集したものなのか、また材料の年齢は同じかどうかという質問にたいし、いずれも新しい葉を用い、またチシマザサ、チマキザサは雪下のものを、ミヤコザサは雪上のものをを用いたという答えがあった。これに対してもしミヤコザサも雪の中の材料を用いれば、透透価、含水量、糖量などの冬期間の変化の傾向は、他のササのものと同じ傾向にありはしないか、また芽の透透価を測ることに意味があるのではないかという意見があった。また移植実験が必要ではないかという見解に対して、比叡山のミヤコザサを四国の海岸に移植すると、いちじるしい形態の変化がおり、たけも短かく、葉の大きさ、形も変わり、茎の基部から枝がでるだけでなく、上部からかなり分枝するようになるという興味深い発言があった。

核心にふれた論議をもちあげるにはいたらないうちに、時間切れとなって論議をうちきった。これについては話題があまりに分散していたという点に一半の責があるが、このことの一つの原因は、従来各研究者が他の分野に無関心でありすぎた点にもあると思う。この意味で異なる分野の研究を知り、それに関連して自分の分野の問題を考え、自分の研究が全体の流れの中のどの位置にあるかを知る機会をもったことは有意義であったと思う。このような機会がしばしば与えられて、離れた分野の知見を総合した活発な論議が展開される機運が生まれることを期待する。(原田市太郎)

話題 2 細胞の構造と機能

10月14日 14.30~17.00

座長 高宮 篤

シンポジウムの2は細胞以下のレベルにおける諸問題というわけで、「細胞の構造と機能」と題する話題を中心にして準備された(準備:高宮 篤・佐藤七郎)。その内容の概略はつぎのとおりであった。

(1) 岡本 尚氏(名大・理・生):細胞膜の構造と機能

細胞膜の研究は現象論から実体論にうつろうとしている。演者はこの方向の見通しにたって研究をすすめる。好塩性クラミドモナスは外液の塩濃度をある限界以下に下げると細胞構造がこわれ、細胞懸濁液の光学密度が減少する。このとき、葉緑体色素の吸収極大における $O.D._{432}$ と懸濁液の光吸収極小における $O.D._{540}$ との比 $r = O.D._{432}/O.D._{540}$ をとると、 r は懸濁液の細胞密度に関係のない、細胞構造の変化の度合を表わす指数となる。細胞の外液に濃度変化を与えると、 r と与えられた溶質濃度 C とのあいだに $r = \text{const.} - k \log C$ の関係がある。数種の溶質について k の相対値を比較した結果、溶質濃度の低下にともなう細胞構造の変化は単なる浸透圧の変化だけによるものでないことが推論された。限界濃度以上ではかなり広範囲の浸透圧変化にたいしても r は一定であるが、これは阻害剤によって顕著に増大する。その効果はおおよそつぎの順になる。

SH 基閉塞剤 > リン酸化阻害剤 > IAA > 呼吸阻害剤

SH 基閉塞剤の存在下に塩濃度を変えると、もうひとつの限界濃度があらわれる。その濃度は細胞の培養塩濃度に依存した。演者は以上の実験成績を考慮し、呼吸阻害と浸透圧調節阻害の時間的経過を追跡した結果、浸透圧調節の osmochemical system は活性中心に SH 基をふくむことを結論し、呼吸系と発酵系・リン酸化系・浸透圧調節系の間のエネルギー転換の仮説を提出し、将来 osmochemical system は収縮性たんぱく質の収縮機構との関連のもとに解析されるべきであると指摘した。

(2) 茅野春雄氏(東大・理・動):カイコ卵の休眠中における糖代謝調整とその機構

昆虫卵の休眠がどのような代謝調整のもとにおこるか、このときにおこる一連の酵素反応と休眠ホルモンの関係はいかなるものであるか、これが本題の目的である。実験の結果、(1)カイコ卵は多量のグリコゲンをふくんでいるが、これが休眠にともなう急激に減少し休眠がおわるとふたたび回復する。(2)グリコゲンの減少は消費によるのではなくソルビットとグリセリンに変わるためである。(3)かかる糖アルコールの生成は休眠卵に特徴的な現象であるということがわかった。この変化は変形された一種の解糖過程であるといえる。ところで、カイコ

卵にはソルビット、グリセリン生成に関してつぎの4つの反応がある。

- 1) $\text{Glucose} + \text{TPNH}_2 \rightleftharpoons \text{Sorbitol} + \text{TPN}$
- 2) $\text{G-6-P} + \text{TPNH}_2 \rightleftharpoons \text{Sorbitol-6-P} + \text{TPN}$
- 3) $\text{Glyceraldehyde} + \text{TPNH}_2 \rightleftharpoons \text{Glycerol} + \text{TPN}$
- 4) $\text{Dihydroxy acetone} - \text{P} + \text{DPNH}_2 \rightleftharpoons \alpha \text{ Glycerol} - \text{P} + \text{DPN}$

この反応を右方にすすめるために要する TPNH_2 DPNH_2 は大部分 pentose-phosphate cycle と解糖系から供給されると推論された。これら cytoplasmic enzyme system で生じた TPNH_2 と DPNH_2 は正常組織ではミトコンドリアの呼吸酵素系によって酸化されるはずであるが、休眠中にはミトコンドリアの電子伝達系のどこかに不通箇所が生じて酸化されず、それが上記の反応をとおして糖アルコール生成をみちびくものであろう。

以上の話題提供をめぐって長時間にわたって活発な質疑応答があった。

このシンポジウムでは動物学者による動物を材料とした研究報告がおこなわれ、前例のない試みなので、はじめその成否が注目されたが、結果的にはなんの不自然も感じさせず、たいへん有益な試みであるという声もあった。今後のシンポジウムのありかたについて重要な参考となるであろう。(佐藤七郎)

話題 3 Species population の問題

10月14日 14.30~17.00

座長 鈴木時夫

定刻 14 時 30 分、座長は開会を宣言し、この話題が、たがいに密接な関係にありながらとかく背中あわせの分野としてとりあつかわれてきた遺伝学と生態学との中間領域の学問的發展に貢献するものであることを強調し、とくに参会者の活潑な討論を要望した。

(1) 福田一郎氏(東京女子大・文理・生)
Population の変遷

オオバナノエンレイソウの Mendelian population の分化の過程について、隔離された小群が、外からは進化圧がはたらいて、内では inbreeding, heterotic vitality がはたらいて分化していくのであ

ろうと説明した。

(2) 石塚和雄氏(岩手大・一般教育・生): 種集団の複合としての植物共同体—八甲田山高田谷地の湿原植生を中心として—

八甲田山のいくつかの距離の近い湿原群で、主要な構成種個体群を共存状態と共同体分析の性質からみて、群集を単位と連続体の中間の位置からながめ species population から community level への研究の一進路を示した。

(3) 黒岩澄雄氏(東大・理・植): 種集団の成立と競争について

ヒマワリの人工群落から縞枯山の自然林にいたるまでの生活集団成立の因果関係についての、種個体群を通じて機構解明へとすすむ一連の研究が成功をおさめつつあることを説明した。

豊富な話題提供の内容は、それ自身立派な特別講演であって、時間はいずれも超過しがちで、参会者も質問をさしひかえて傾聴した。

総合討論にはいるに先だち、座長は話題提供者相互の質問討論によって、共通の論点をひき出そうと試みたが成功せず、参会者の質問にはいった。越智、小清水両氏の質問のあと、宝月氏が立ち、この話題がシンポジウムにえられた経緯につき、計画者として発言があり、宮脇昭氏からこれに対する質問があった。座長は、これら総合討論当初の発言中から重要な問題を取りあげて高揚すべきであったがそのことなく、続いて一般の質問にはいったため、最後まで中心的論点を打ち出すことができず、質問は種子集団のこと、種名のことなど黒岩氏に集中するかのごとくみえた。

当初の不手ぎわを回復すべく、また話題提供者全部、とくに遺伝学者の発言を喚起するために参会者および福田氏に対し座長からとくに発言を要望した。ここにおいて小野記彦氏、福田氏の発言あり、生態学者の質問も福田氏に対し集中した。座長はこの機会をとらえ、一挙に生態学と遺伝学共通の話題をひき出そうと企図して、オオバナノエンレイソウ個体群の生育地ならびに、その従属する植物社会につき、環境の人為攪乱が Mendelian population に対して進化圧ともいうべきある種の作用をへて遺伝子に変化を与えるのではないかというような発言をした。予定時間はすでに約 10 分をあますばかりであったが、これに対して渡辺定元氏から、オ

オバナノエンレイソウの生育地についての説明あり
福田氏からも函館山その他における生育地の説明が
なされた。ここで集団遺伝学研究者の登場がもっと
多く期待されたが、指名する機会を失した。

問題点はなかなかしぼられなかったが、討論は白
熱して座長は 15 分間の時間延長を提案、少なくと
も、species population が遺伝学・生態学・分類
学・進化学の各方面から、もっと重視してとりあげ
られなければならないという印象を全員がもつとい
う方向にむかった。しかし、提起された三つの立場
の総合という方向に討論が展開しはしたが、はっき
りと結論づけられるには至らなかった。

これについては、座長がこの問題に興味をもって
はいたが全分野にわたる安定した識見に不足したこ
と、話題提供者が完全なる講演という点にあまり努
力しすぎて短い時間に共通の討論に発展する余地を
残さなかったこと、これに対して一般参会者がどち
らかという資料の検討不足で反射的な思いつき質
問を多く出したということ、などが反省される。し
かし、話題提供者が権威ある事実の裏づけをもって
講演されたため、ややもすれば空論に流れた過去の
シンポジウムに対して画期的な進歩を示し、また座
長の要望のごとく活発に討論がおこなわれた点は成
功といってよいとおもわれる。(鈴木時夫)

話題 4 細胞と組織の分化

10 月 15 日 14.30~17.00

座長 前川文夫

(1) 林 俊郎氏(東大・教養・生): 高等植物に
おける細胞培養とその応用

高等植物の細胞を遊離状態で培養することは、
F.C. Steward が 1949 年に成功して以来、12 年
間にかかなりの進歩が見られる。方法的には試験管ま
たはフラスコに液体培地とともに組織片を入れ、回
転または振とうすることによって得られるが、培地
にはココナットミルクを加えるのが普通である。一
般に、毎分 1~60 回の低速では遊離細胞のほか、
カルス状のものや小細胞群も生じるが、毎分 200~
300 回でカルス状のものはなくなる。次に遊離した
単一細胞をとり出し増殖させることは、ちょうど旺盛
に細胞が増殖中の培地を用いるか、または増殖中

の組織上にろ紙をおきその上にのせる方法 (nurse
culture) を用いるかして成功している。

演者は、ヒマワリの茎から得た典型的な crown
gall の組織を用い、振とうまたは回転培養をおこ
ななしたところ、本来は分化しないこの組織が発根す
ることがわかった。しかし発根能力は、植物体から
ぎりとりて 8 か月以内に限られ、その後は分化の能
力を失った。また発根したものも寒天培地に移すと
dedifferentiation してもとの典型的な腫瘍組織にも
どることが観察された。

この講演に対し、crown gall の定義、crown gall
と tissu anergié との差異、振とう培養で組織がばら
ばらになる機構などについて討論がおこなわれた。

(2) 佐藤七郎氏(東大・理・植): 植物の組織分
化を研究する方法について

従来、植物の組織の分化を研究するのに用いられ
てきた方法を大別すると、生化学的方法と組織化学
的方法とがある。前者は組織内の半径方向の分化を
いちおう無視して、縦軸の方向の分化のみに注目し
軸の先端(たとえば根端)からの距離と、その部分
の酵素活性、細胞分裂頻度などとの対応をつけて分
化の物質的基礎をさぐるとうする方法である。この
方法は定量的である点を強みとするが、軸の内部分
化を無視するところに決定的な弱みがある。後者は
器官の内部分化を重視し、これと酵素活性などとの
対応をつけようとするものであるが、これは前者と
は逆に、定量的でない欠点をもつ。以上の批判のう
えにたって、演者は細胞の質的差異を無視せずに、
しかも分化機構の定量的な実験解析をおこなうため
には、組織を macerate し、同質の細胞を mass と
して集める技術確立しなければならないと結論し
この方法の分化研究上における意義を強調した。
さいごにこの方法が技術的に可能であることを数枚
のスライドによって示した。この講演に対して、そ
のような方法の意義は演者が強調するほど重大でな
い、またそのような技術が達成されたとしても、そ
の先どのような実験をして組織の分化を研究してい
くかが問題である、などの批判がだされて賛否両論
に分かれ、活発な討論がおこなわれた。(駒嶺穆)

見学旅行の記録

10月16日(月) 参加者 66名.

午前9時6分上野駅発, 11時28分水戸駅着.
これからバス2台に乗って見学コースに入る. 水戸旧城三の丸にある県立原子力館で原子炉, 原子燃料レモ・コン装置などを見て説明をきく. ここから坦々たる原研道路を飛ばす. まわりはサツマイモ畑とクロマツの防風林. 午後1時, 原子力研究所につきまず食堂で食事. ここは水戸藩以来育成されたクロマツの大砂防林中にあって, 総面積は約330ha, 林を開いたところに近代的な研究室, 発電炉などが規則正しく並んで偉容を誇っている. 1号炉, コバルト照射室を見学した後, 海岸近くの汚染物処理場, 動力炉(建設中)を見る. 松林をへだててコールダーホール改良型炉の建設現場も見える. 2時40分ふたたびバス. 車窓から原子燃料公社製錬所, 村松の虚空蔵さんをちらりと見て那珂湊海岸着. 海岸に規則正しく隆起する白堊紀化石層を見学. 茨城大齊藤講師の説明に耳をかたむける. 時間がなくてあわ

ただしいのが残念, コハマギクが点々と咲いている. バスは那珂川にかかる高脚の海門橋を渡り, 大洗海岸にて小休止. 砂浜と岩, おとぎ話風の小水族館あり. 付近の松林は先日の台風で少しいたんでいる. バスを飛ばし, 4時54分水戸発上野行の電車にやっとまにあう. 8時前上野駅にて解散.(見学係)

支 部 通 信

九州支部

第66回例会(昭和36年9月30日 福岡女子大において)

鈴木時夫・柏 宏人(大分大学芸生物): 屋久島の森林土壌について, 鈴木時夫(大分大学芸生物): Braun-Blanquet, J., Pallmau, H., und Bach, R. 著“Pflanzensoziologische und bodenkundliche Untersuchungen im schweizerischen Nationalpark und seinen Nachbargebieten II. Vegetation und Böden der Wald- und Zwergstrauchgesellschaften [Vaccinio-Piceetalia]”の抄読

Error

One line was dropped between 17th and 18th lines in the key on page 327 of Vol. 74 Nos.

877-878. It runs as follows:

9. Leaves capillary, involute-margined, less than 1 mm wide. 8. var. *Fernaldiana*.

